Serotonergic modulation of sexual behavior in male rats and men

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Serotonergic modulation of sexual behavior in male rats and men

Serotonerge modulatie van seksueel gedrag
bij mannelijke ratten en mannen

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voor mijn oma's
en mijn ouders

Contents

Chapter 1

Introduction

SEXOLOGY IS A MULTIDISCIPLINARY SCIENCE. When looking for a suitable definition for ejaculation, different disciplines focus on different aspects of ejaculation. Van de Velde, a Dutch gynaecologist early this century and writer of the first Dutch book on sexology for lay people, *Het Volkomen Huwelijk* (1923, The ideal marriage), called it "het doel, de culminatie en het eigenlijke slot van de geslachtsgemeenschap" (the goal, the culmination, and the actual conclusion of the sexual intercourse). The sexologists Masters and Johnson (1966) stated: "it can be identified by a chain of specific physiologic reactions and by correlated patterns of subjective progression." The urologist Kedia (1983) called it "an expulsion of seminal fluid to the exterior of the organism by the rhythmic contractions of the perineal muscles. It constitutes an essential link in the behavior chain leading to reproduction and perpetuation of the species." De Beauvoir (1949), novelist and feminist wrote: "in ejaculation the male rids himself of certain discomforting secretions; he obtains a complete relief, following sex excitement, which is unfailingly accompanied with pleasure." The clinical psychologist Zilbergeld (1992) called it "a total body response, not just something that happens in the crotch." Finally, in a textbook of physiology, ejaculation is explained as "sympathetic impulses that leave the cord at L-1 and L-2 and pass to the genital organs through the hypogastric plexus to initiate emission" (Guyton, 1986).

In my opinion, ejaculation is an intermediate between an eruption of pleasure and evolutionary necessity, and a result of a cascade of physiological mechanisms, strictly co-ordinated by the central nervous system and almost inevitably associated with an experience of orgasm. Although ejaculation is usually associated with pleasant sensations, it could also cause serious problems in the relationship as well. A well-known example is the most common male sexual dysfunction, premature ejaculation (*ejaculatio praecox*), a condition which affects

up to 29-40% of the adult males (Althof, 1995, Metz *et al.*, 1997). This sexual dysfunction can cause problems ranging from an unsatisfactory sex life, feelings of guilt, depression, to undesired childlessness. Hopefully, the experiments described in this thesis contribute to an increase in the knowledge of neuronal control of ejaculation, and therefore be of help in finding a medical treatment for premature ejaculation.

AIM OF THIS THESIS

The aim of this thesis is to investigate the role of serotonergic agents (5-HT_{1A} receptor agonists, serotonin reuptake inhibitors) in the control of ejaculation in the male rat and in human males. Central questions are:

1. Is it possible to make an animal model for premature ejaculation with selective 5-HT_{1A} receptor agonists such as 8-OH-DPAT and flesinoxan?
2. Is it possible to stimulate sexual behavior in male rats with low or impaired sexual behavior (middle-aged rats, neonatally ATD-treated rats) with the selective 5-HT_{1A} receptor agonist 8-OH-DPAT?
3. Is premature ejaculation in humans (partially) due to hyperaesthesia of afferent skin receptors in the penis?
4. Can serotonin reuptake inhibitors, such as clomipramine and fluoxetine, have beneficial treatment properties in human males with premature ejaculation?

In chapter 2, some basic concepts of neuropharmacology will be explained. Subsequently, details are given about the neurotransmitter serotonin, and a classification is presented of the serotonergic receptors.

Part 1 of this thesis describes the animal studies. In chapter 3, normal sexual behavior of the rat will be explained. Also, a review of the literature will be given on the subject of serotonergic control of male rat sexual behavior. In chapter 4, the influence of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT on masculine sexual behavior in male and female rats is described. In chapter 5, it was investigated whether or not testosterone was required for the stimulatory properties of 8-OH-DPAT on sexual behavior in male rats. Finally, in chapter 6, a

comparison was made of the stimulatory properties of two selective 5-HT_{1A} receptor agonists, 8-OH-DPAT and flesinoxan on male rat sexual behavior, both in control rats and in rats with impaired sexual response, due to neonatally treatment with ATD.

In Part 2 the human studies are described. In chapter 7, premature ejaculation is defined and a review is given about the historical treatments, the currently available psychosexual therapy and the pharmacotherapies. In chapter 8, penile sensitivity threshold to vibrotactile stimulation was compared in men with premature ejaculation and controls. Finally, pharmacotherapy for premature ejaculation was investigated with the serotonin reuptake inhibitors clomipramine (chapter 9) and fluoxetine (chapter 10) in prospective, double-blind, placebo controlled, crossover studies with patients and controls.

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Chapter 2

Serotonin receptors

BASICS OF NEUROPHYSIOLOGY

Receptors

The term "receptor" can be used to indicate any clear-defined target molecule in the cell or cell membrane with which a hormone, neurotransmitter, drug molecule or any other substance has to combine in order to elicit its specific effect (Rang and Dale, 1987). In general, the specific substance reacts with the receptor, forming a complex. This complex activates a reaction in the cell or cell membrane and therefore enables the cell to exert its function. Certain substances (agonists) can be said to activate the receptors, and others (antagonists) may combine with the same site without causing activation. Receptors form a key part of the system of chemical communication that all multicellular organisms use to co-ordinate the activities of their cells and organs. Without them we would be no better than a bucketful of amoebæ (Rang and Dale, 1987).

Synapses

Nerve signals are transmitted from one neuron to the next through interneuronal junctions called synapses. The synaps consists of a presynaptic terminal (or bouton), a synaptic cleft measuring approximately 200-300 Ångstroms, and the soma of the next neuron. In the presynaptic terminal, a chemical substance is secreted into the synapse, diffuses to the soma of the next neuron, where it acts on a specific receptor in the membrane to excite, to inhibit, or to modify the neuron's sensitivity in some other way (Guyton, 1986).

Neurotransmitters

The chemical substance for communication between neurons are called neurotransmitters. The synthesis of neurotransmitters occur partially or totally in the cytoplasm of the presynaptic terminals, where they are stored in the multiple

transmitter vesicles. When an adequate impulse (action potential) arrives at the presynaptic terminal, the vesicles release the transmitter content into the synaptic cleft (exocytosis). Subsequently, it is possible for the neurotransmitter to diffuse to the soma of the next neuron and bind with a receptor. There are three ways to disperse the neurotransmitter from the synaptic cleft. 1. diffusion of the transmitter out of the cleft into surrounding fluids. 2. metabolic degrading within the cleft itself by enzymatic destruction (for example, serotonin is disintegrated by monoamine oxidase). 3. transmitter re-uptake: active transport back into the presynaptic terminal itself for reuse. Over 30 different neurotransmitters have been described so far, including well-known substances like acetylcholine, (nor)epinephrin, dopamine, serotonin, GABA, and many more (Guyton, 1986).

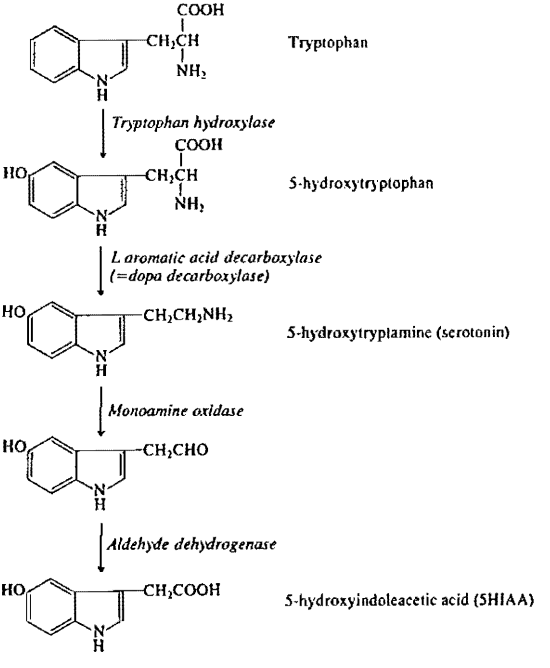

FIGURE 2.1. Metabolism and degeneration of serotonin

SEROTONIN

Serotonin (5-hydroxytryptamine, 5-HT) is a substance found in different places in the body (see below). The biosynthesis of 5-HT is depicted in figure 2.1. Serotonin is present in the food, but is mostly metabolized before entering into the bloodstream. In chromaffin cells and neurons, but not in platelets, tryptophan is converted into 5-hydroxytryptophan by the action of tryptophan hydroxylase. The 5-hydroxytryptophan is then carboxylated to 5-hydroxytryptamine, also known as serotonin. The same decarboxylase enzyme acts on many other substrates, and is also involved in the synthesis of catecholamines and histamine (Rang and Dale, 1987). Degradation of serotonin occurs mainly through oxidative deamination, catalysed by monoamine oxidase, this is followed by oxidation to 5-hydroxy-indoleacetic acid (5-HIAA). 5-HIAA is excreted in the urine, and can be used as an indicator of serotonin production in the body (Rang and Dale, 1987).

Distribution of serotonin

About 90% of the serotonin in the human body is situated in the wall of the intestine, more specifically in the chromaffin cells (Rang and Dale, 1987), which play a role in the distribution of catecholamines (Junquiera and Carneiro, 1983). Serotonin is also present in the blood platelets, and is released at sites of tissue damage. Finally, serotonin is found in the central nervous system, where it acts as a neurotransmitter (Rang and Dale, 1987).

Pharmacological effects of serotonin

In the gastrointestinal tract, serotonin causes increased motility and contraction. In the larger blood vessels, both arteries and veins, serotonin causes usually constriction. In the microcirculation it causes constriction in the arterioles, constriction in the venules and an increased permeability of the capillaries, thus encouraging the formation of tissue fluid (Rang and Dale, 1987). The effects of serotonin as a neurotransmitter in the brain is described below.

Serotonin receptors

In 1948, Page discovered that when blood is allowed to clot the vasoconstrictor substance released was serotonin (5-HT; Rang and Dale, 1987). In 1957, 5-HT receptors were divided into D (dibenzyline-sensitive muscultotropic)-receptors and M (morphine-sensitive neurotropic)-receptors (Gaddum and Picarelli, 1957).

However, from time to time it was reported that some effects of 5-HT, for example vasoconstriction of the carotid vascular bed, were mediated by non-M and non-D receptors (review: Saxena and Ferrari, 1992). Subsequently, the 5-HT_1 and the 5-HT_2 receptors were identified (Peroutka and Snyder, 1979). Since the 5-HT receptors were being referred to by various names (D, M, 5-HT_1, 5-HT_2, S_1, S_2), a uniform terminology was needed. In 1986, Bradley and co-workers proposed a general framework for the characterisation and nomenclature of the 5-HT receptors (Bradley *et al.*, 1986). The 5-HT receptors were classified into three main categories: 5-HT_1-like (corresponding to some D-receptors and 5-HT_1 binding sites), 5-HT_2 (corresponding to most D-receptors and 5-HT_2 binding sites) and 5-HT_3 (equivalent to M-receptors; Saxena, 1994).

To date, more than 50 different 5-HT receptor subtypes have been described (Saxena, 1994). They include 7 main groups (5-HT_1 to 5-ht_7), and most of these groups are divided into subgroups (for example, 5-HT_1 is subdivided into 5-HT_{1A} to 5-ht_{1F}). The lower case letters (for example, 5-ht_{1F}) are used for recombinant receptors with little knowledge of operational characteristics and the higher case letters (for example, 5-HT_{1A}) are used for reasonably well characterized native receptors (Hoyer *et al.*, 1994). There is also an "orphan" category of serotonin receptors which include receptors that await further characterization (Saxena, 1994).

5-HT_{1A} RECEPTORS

Location

The 5-HT_{1A} subtype receptors are somatodendritic and terminal autoreceptors in the central nervous system, and are mainly found in the hippocampus. Other brain areas include the septum, some of the amygdaloid, and raphe nuclei, particularly the dorsal raphe (Marcinkiewicz *et al.*, 1984, Radja *et al.*, 1991, Marsden and Kendall 1992). There is evidence that 5-HT_{1A} receptors are located mainly postsynaptically (Hoyer *et al.*, 1994).

Action

Facilitation of the 5-HT_{1A} receptors results in an inhibition of neuronal firing of rat (dorsal) raphe neurons, and subsequently in a fall of extracellular 5-HT in the striatum and ventral hippocampus (Marsden and Kendall 1992, Kreiss and

Lucki 1994). Thus, administration of a 5-HT_{1A} receptor agonist (for example 8-OH-DPAT or flesinoxan) will result in reduction of the 5-HT synthesis, release, and electrical activity (De Montigny and Blier, 1992; see figure 2.2).

When rats are exposed to relatively high doses of a 5-HT_{1A} receptor agonist, a behavioral syndrome is induced, which is called the "5-HT syndrome." It is characterized by flat body posture, reciprocal forepaw treading ("piano playing"), head weaving, abducted hindlimbs, occasional tremor in the forebody and lower lip retraction (Arvidsson *et al.*, 1981, Tricklebank 1985, Berendsen *et al.*, 1989). Other effects of these 5-HT_{1A} receptor agonists include hypophagia, anxiolysis and decrease in blood pressure and heart rate (Hoyer *et al.*, 1994). The effects of 5-HT_{1A} receptor agonists on male rat sexual behavior are reviewed in chapter 3 and described in chapters 4-6 of this thesis.

5-HT_{1A} receptor agonists

In 1981, Arvidsson and co-workers described a compound, which is now assumed to be the gold standard of 5-HT_{1A} receptor agonists: 8-hydroxy-2-(di-n-propylamino)tetralin, or 8-OH-DPAT (Arvidsson *et al.*, 1981). It has a high affinity for the 5-HT_{1A} receptor (K_i=2.8 nM). The next highest affinity is for alpha$_1$-adrenergic receptors (K_i=300 nM), a difference of a factor of more than 100 (Schipper *et al.*, 1991). 8-OH-DPAT is not suitable for human studies because only a poor bioavailability can be reached with oral administration (R. van Oorschot, *personal communication*)

Another selective 5-HT_{1A} receptor agonist is flesinoxan, a phenylpiperazine derivative. The chemical structure is (+)-N-[2-[4-(2,3-dihydro-2-hydroxymethyl-1,4-benzodioxin-5-yl)1-piperazinyl]ethyl]-4-fluorobenzamide hydrochloride. It has a high affinity for the 5-HT_{1A} receptor (K_i=1.7 nM). The next highest affinity is for dopamine-D_2 receptors (K_i= 140 nM), a difference of a factor 82. Flesinoxan and 8-OH-DPAT have a comparable potency and selectivity for the 5-HT_{1A} receptor (Schipper *et al.*, 1991). In human studies, flesinoxan is tested for its antidepressant and anxiolytic properties.

It should be noted that interpretation of the behavioral experiments with 5-HT_{1A} receptor agonists are limited by the lack of selective receptor antagonists (Ahlenius and Larsson, 1984). Certain beta-blocking agents like (-)pindolol and (-)alprenolol do have some specificity in this regard, but so far it has not been possible to separate beta-blocking from 5-HT_{1A} blocking properties (Ahlenius and Larsson, 1989). With the opoid receptor agonist naloxone an inhibition of the

facilitatory effects of 8-OH-DPAT on male rat sexual behavior was found (Ågmo *et al.*, 1989), but since these two agents act on different systems of receptors, the results are difficult to interpret.

OTHER SEROTONIN RECEPTORS

5-HT$_{1B}$ receptors

The 5-HT$_{1B}$ site has been more difficult to characterize since it is not present in the guinea-pig, cow, chicken, turtle, frog, or human brain, but only in the rat and mouse brain (Fontez Ribeiro, 1991). The 5-HT$_{1B}$ receptors may function as autoreceptors at nerve terminals of the rat brain (Engel *et al.*, 1986). Functionally, 5-HT$_{1B}$ receptor agonists induce some components of the 5-HT syndrome (see above), such as head-weaving and hypophagia (Kennet and Curzon, 1988). In a behavioral experiment with the 5-HT$_{1B}$ receptor agonists RU 24969, TFMPP and mCPP, Fernández-Guasti and co-workers (1988) found an increase in mount frequency, intromission and ejaculation frequencies in male rat sexual behavior.

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PART 1

ANIMAL STUDIES

Chapter 3

5-HT_{1A} receptors and sexual behavior of the male rat*

THE ALBINO LABORATORY RAT (*Rattus norvegicus*) is the most commonly used mammal in animal models of sexual behavior. The male rat sexual behavior consists of three very distinct and easily recognizable elements: mounts, intromissions and ejaculations. In the first part of this chapter, the different elements of sexual behavior are presented in more detail.

Several neurotransmitters are reported to have modulating (stimulating or inhibiting) properties in the control of ejaculation. A review of the literature is given on serotonin and its subreceptor 5-HT_{1A}, dopamine and various other neurotransmitters in the second part of this chapter.

SEXUAL BEHAVIOR OF THE RAT

Male rat sexual behavior

The mating pattern of the normal male rat when tested with an estrous female consists of repeated mounts and intromissions culminating in ejaculation (figure 3.1). The ejaculation is followed by a period of 4 to 5 minutes during which time the rat remains refractory to sexual stimulation (post-ejaculatory interval). The sexual activity is thereafter resumed with a new series of mounts and intromissions followed by ejaculation (Ahlenius and Larsson, 1987). A typical pattern of normal male rat sexual behavior, when tested with an estrous female, is given in figure 3.2.

Operational definitions of different male rat sexual behaviors used in this thesis (Bakker, 1996):

-*Mounts (Mts)*. Rat ascends receptive conspecific (usually estrous female rat in lordosis) from the rear, and makes thrusting movements with the pelvis.

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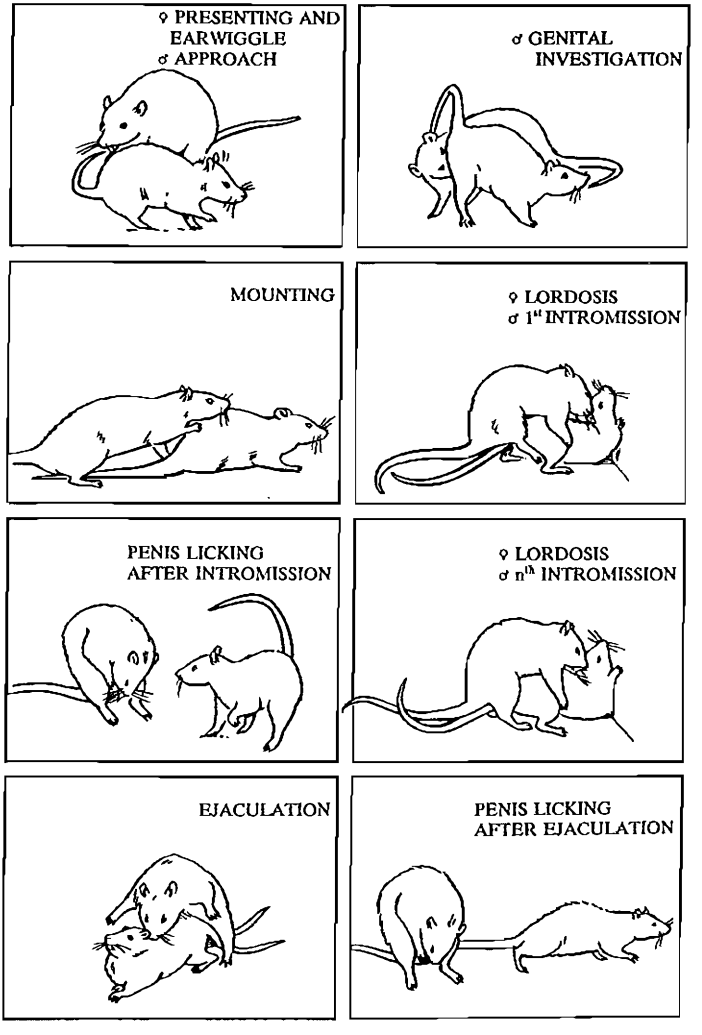

FIGURE 3.1. Different components of rat sexual behavior (*From*: Slob and Van der Werff ten Bosch, 1997)

-*Intromissions (Int)*. Same as mounts, but immediately followed by one deep pelvic thrust, when the penis is penetrated into the vagina. This penetration lasts for about 200-400 msec.

-*Ejaculation (Ejac)*. Culmination of vigorous intravaginal thrusting accompanied by the arching of the male's spine and often lifting the forepaws off the female prior to withdrawal.

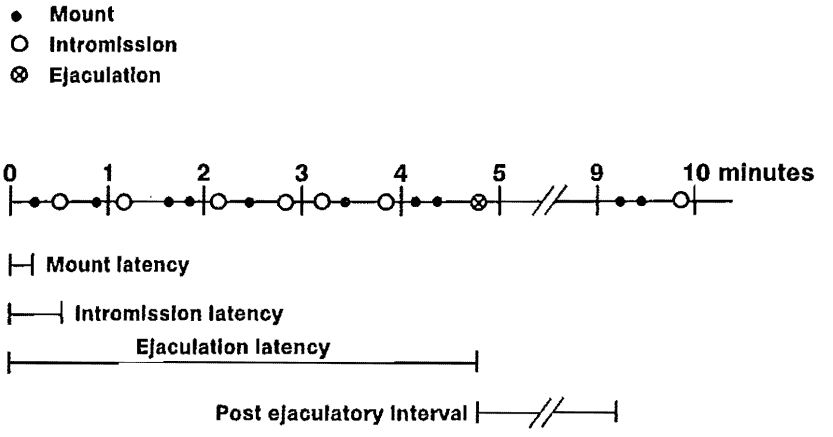

FIGURE 3.2. Typical pattern of normal male rat sexual behavior

-*Mount latency (ML).* Time from start of behavioral testing to first mount. In normal, well-trained rats this usually lasts only 3-6 sec.

-*Intromission latency (IL).* Time from start of behavioral testing to first intromission. Some authors use for IL time from first mount to first intromission.

-*Ejaculation latency (EL).* Time from start of behavioral testing to first ejaculation. In normal, sexually experienced rats this lasts about 3-4 min. Some authors use for EL time from first mount (or first intromission) to first ejaculation.

-*Post-ejaculatory interval (PEI).* Synonym: refractory period. Time from ejaculation to first following sexual behavior. Usually this lasts about 5 min.

Other masculine sexual behaviors of the rat comprise *exploration* of the (female) conspecific, usually by sniffing at the genitals, *grooming* the own genitals, usually after intromission or ejaculation and *vocalization* at 22 kHz in the post-ejaculatory interval (the so-called *post-ejaculatory song*). The exploration, grooming and vocalization are behaviors that were not investigated in detail in the experiments described in this thesis.

Female rat sexual behavior

Typical estrous female rat sexual behavior consists of presentation, ear wiggling and lordosis. The ovarian cycle of the female rat lasts 4-5 days. That

means that once every 4-5 days, she will be in estrus and concomitantly receptive to sexual approaches of a male conspecific (Bermant and Davidson, 1974, Slob and Van der Werff ten Bosch, 1997). During the night of ovulation the female rat is anxious to copulate for several hours. If there is an opportunity, she will actively seek a potential male partner, which she recognizes as such through his dihydrotestosterone-dependent odor, which is known as pheromone (Vreeburg and Ooms, 1985). To attract attention of the male conspecific, she will hop and dart, shake her head rapidly (*ear wiggling*), and *present* herself with an arched back and rear end directed to the male (Beach, 1976). The female may also mount, but only if a female partner or a sluggish male is present (Beach, 1968). In response to genital sniffing and licking of the male partner, the fully estrous female will stretch her back by raising her head and perineal region (*lordosis*) which incites and enables the male to mount her. Under natural circumstances the mated female will become pregnant and give birth to a litter of about 10 pups 21 or 22 days later. A few hours after parturition she will come into estrous again (postpartum heat), and if a male is available, she will mate and become pregnant again (Slob and Van der Werff ten Bosch, 1997)

In our experiments, we used ovariectomized female rats, that were brought into behavioral estrus with subcutaneous injections with estradiol and progesterone preceding the behavioral experiments.

5-HT AND MALE RAT SEXUAL BEHAVIOR

Serotonin and ejaculation

Ever since the late sixties, serotonin has been known for its involvement in male rat sexual behavior. It is generally assumed that central 5-HT has an inhibitory role in the neural control of masculine sexual behavior in the rat (Ahlenius *et al*, 1980). The inhibitory action is mediated via 5-HT$_2$ receptors and the stimulatory actions seen after agents acting on other receptors (e.g. 5-HT$_{1A}$) is probably due to inhibition of the release of endogenous 5-HT (Wilson, 1993). A decrease in 5-HT neurotransmission decreases the number of intromissions preceding ejaculation and shortens the time to ejaculation, whereas an increase in central 5-HT neurotransmission produces the opposite effect (Ahlenius and Larsson, 1989). Early behavioral tests were performed with the tryptophan hydroxylase inhibitor para-chlorophenylalanine (pCPA) (Koe and Weismann,

1966), alone or in combination with an inhibitor of monoamine oxidase, with facilitated the ejaculation frequency in male rats (Ahlenius *et al.*, 1971, Salis and Dewsbury, 1971). This effect was antagonized by administration of the 5-HT precursor 5-HTP (Södersten *et al.*, 1976). Experiments with intracerebral injections of the neurotoxine 5,7-DHT (Larsson *et al.*, 1978), and electrolytic lesions of the raphe nuclei of the brain stem (Ahlenius and Larsson, 1987) showed facilitation of the male rat sexual behavior. Taken together, these observations suggest that central 5-HT has an inhibitory role in the mediation of male rat sexual behavior (Ahlenius *et al.*, 1980).

8-OH-DPAT AND MALE RAT SEXUAL BEHAVIOR

In the first publication on the effects of 8-OH-DPAT on male rat sexual behavior, Ahlenius and coworkers (1981) described a dose-dependent reduction in the number of intromissions preceding ejaculation (medians from about 8 with saline, to 2 with 4 mg/kg 8-OH-DPAT), a shortening of ejaculation latency (medians from about 7 min with saline to less than 2 min with 4 mg/kg 8-OH-DPAT) and a reduction in the post-ejaculatory interval with 8-OH-DPAT treatment. Also, with 1-4 mg/kg 8-OH-DPAT, some animals ejaculated at the first intromission (Ahlenius *et al.*, 1981). In analogy to what is described in the human, this phenomenon could be called "premature ejaculation." The typical pattern of a normal male rat sexual behavior and male rat sexual behavior influenced by 8-OH-DPAT is shown in figure 3.3.

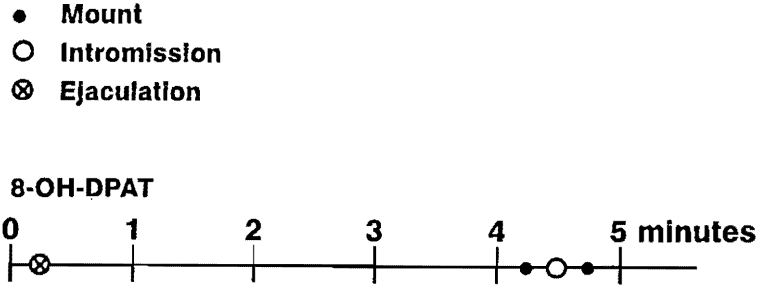

FIGURE 3.3. Typical pattern of male rat sexual behavior, influenced by 8-OH-DPAT

Other groups have confirmed the findings of Ahlenius and collaborators and added some new facts to the knowledge of the effects of 8-OH-DPAT on male rat sexual behavior. Morali and Larsson (1984) found that the pelvic thrusting pattern of the male rats was not altered by 8-OH DPAT.

Schnur and coworkers (1989) performed an *ex copula* genital reflex test in which spontaneous erections and ejaculations are investigated. Administration of 8-OH-DPAT caused an inhibition of spontaneous erections and ejaculations. In a mating test in the same study, they described 4 (of 13) rats, who ejaculated extravaginally when given 1.0 mg/kg 8-OH-DPAT. Furthermore, a trend towards dose-dependent reduction in copulatory plug weight with 8-OH-DPAT was described.

Brand *et al.* (1991) investigated the effect of 8-OH-DPAT on the sexual behavior in male rats, perinatally treated with the aromataze inhibitor 1,4,6-androstatriene-3,17-dione (ATD). This compound is known to impair sexual behavior (Vreeburg *et al.*, 1977, Davis *et al.*, 1979). Treatment with 8-OH-DPAT compensated, at least partially, the perinatal effects of ATD on adult partner preference behavior and on ejaculatory behavior (Brand *et al.*, 1991).

The effects of 8-OH-DPAT on male rat sexual behavior disappear when the rats are chronically treated with the compound. Johansson and coworkers (1990) administered daily injections of .033 to 0.1 mg/kg 8-OH-DPAT for 8 to 15 days and tested the male rats 15 min after drug administration. There was a decrease in stimulatory properties to male rat sexual behavior of 8-OH-DPAT, presumably due to tolerance and decreased sensitivity (Johansson *et al.*, 1990).

To investigate if 8-OH-DPAT exerts its effects centrally or peripherally, various studies have been published. For example, Dahlöf and coworkers (1988) desensitized the rat penis by surgical dissection of the pudendal nerve. Subsequent administration of 8-OH-DPAT normalized the inhibited ejaculatory response. Lee and collaborators investigated the effects of intrathecal administration of 8-OH-DPAT on male rat sexual behavior. They found a reduced ejaculation latency, intromission frequency and inter-intromission interval (Lee *et al.*, 1990). In another study, 8-OH-DPAT was selectively injected in the preoptic area, the nucleus accumbens and the dorsal raphe nucleus, and it caused a decrease in mounts and intromissions prior to ejaculation, and in ejaculation latency. No effect was found on the post-ejaculatory interval (Fernández-Guasti *et al.*, 1992). These and other studies all point towards a central action of 8-OH-DPAT.

The effects of 8-OH-DPAT on male sexual behavior are almost exclusively investigated in the rat. Only few studies have appeared on other species. Pomerantz and coworkers described a facilitation of sexual behavior in rhesus monkeys by reduction of the number of intromissions prior to ejaculation and a reduction of ejaculation latency with low doses (5 or 10 μg/kg) 8-OH-DPAT (Pomerantz *et al.*, 1993a) With higher doses (0.1 or 0.2 mg/kg) 8-OH-DPAT and without the possibility of physical interaction with a sexually receptive female monkey, an inhibition of the occurrence of spontaneous erections was found (Pomerantz *et al.*, 1993b). In male ferrets treated with estradiol benzoate, 8-OH-DPAT inhibited duration of the neck grip and had no effect on other male sexual behaviors (Paredes *et al.*, 1994). In gonadally intact male rabbits, 1 mg/kg 8-OH-DPAT eliminates sexual behavior whereas lower doses have no effect (Ågmo *et al.*, 1991). Thus, 8-OH-DPAT has different effects on sexual behavior in different species. This makes extrapolation of the results of male rat experiments with the selective 5-HT_{1A} receptor agonist to the human difficult.

Although above effects are sufficiently investigated and described, some questions remain. What is the effect of 8-OH-DPAT on the sexual response in "sluggish" middle-aged male rats? If adult male rats ejaculate "prematurely" (i.e. at the first or second intromission), do they ejaculate intravaginally? Do estrous female rats prefer the vicinity of 8-OH-DPAT-treated males, or do they prefer untreated male conspecifics? What is the effect of 8-OH-DPAT on the masculine sexual behavior of female rats? In chapter 4 experiments are described to find an answer to these questions.

The group of professor J.M. Davidson from Stanford University, CA did some studies on the relationship between circulating testosterone levels and male sexual behavior in rats without and with the facilitating agent yohimbine (Damassa *et al.*, 1977, Clark *et al.*, 1984). This inspired us to investigate whether or not it is necessary to have measurable serum levels of testosterone find the stimulatory effects of 8-OH-DPAT on sexual behavior in male rats. In chapter 5 this experiment is described.

8-OH-DPAT and female rat sexual behavior

In intact female rats, 8-OH-DPAT depresses lordosis behavior (Mendelson and Gorzalka, 1986, Uphouse *et al.*, 1991). This effect does not disappear when the female rats are chronically treated with 8-OH-DPAT (Johansson and Meyerson, 1991). Although other serotonergic agents also affect lordosis behavior in female

rats, the inhibition is mainly mediated by 5-HT$_{1A}$ receptor agonists (Gorzalka *et al.*, 1990, Ahlenius, 1993).

Mendelson and Gorzalka (1986) investigated the masculine sexual behavior of female rats when put in the vicinity of an estrous female rat. Administration of 8-OH-DPAT in ovariectomized female rats, chronically treated with testosterone propionate (to stimulate masculine sexual behavior in these females) resulted in a discrete, but statistically significant increase in mount frequency in these female rats. In chapter 4, experiment 4.4 we investigated masculine sexual behavior in the female rat, without and with chronical treatment with testosterone propionate.

FLESINOXAN AND MALE RAT SEXUAL BEHAVIOR

Flesinoxan, also a selective 5-HT$_{1A}$ receptor agonist, is reported to react in a similar way as 8-OH-DPAT in behavioral and pharmacological studies with male rats. Mos *et al.*, (1990) administered various serotonergic drugs, including 8-OH-DPAT and flesinoxan. It resulted in an increase of ejaculation frequencies, and in a decrease of ejaculation latency and mount and intromission frequencies prior to ejaculation. Compared to flesinoxan, the effects of 8-OH-DPAT appeared to be "stronger." Unfortunately, the authors gave no details on how many rats ejaculated at the first or second intromission with the different drugs, and only the first ejaculation series was described.

Ahlenius *et al.* (1991) found an increase of ejaculation frequencies, and a decrease of ejaculation latency and mount and intromission frequencies prior to ejaculation. They concluded that flesinoxan enhances sexual behavior in the male rat in a similar way, as 8-OH-DPAT, with flesinoxan being about an order of a magnitude less potent (Ahlenius *et al.*, 1991). However, when critically reading the study, no "premature ejaculations" were described (i.e. ejaculation at the first or second intromission).

To investigate the effects of flesinoxan on male rat sexual behavior, and to compare the effects of 8-OH-DPAT and flesinoxan, a study was performed that is presented in chapter 6.

VARIOUS 5-HT_{1A} RECEPTOR AGONISTS AND MALE RAT SEXUAL BEHAVIOR

In a study with the 5-HT_{1A} receptor agonist buspirone, Mathes and coworkers (1990) found a significant decrease in latency to first ejaculation in male rats, when tested with an estrous female. The number of intromissions prior to ejaculation was significantly reduced after buspirone treatment (medians: from 6 (vehicle) to 4 (1-4 mg buspirone IP)). Unfortunately, no information was given about the number of animals that ejaculated with the first or second intromission after buspirone treatment. No difference was found between intrathecal and intraperitoneal administration of buspirone (Mathes *et al.*, 1990).

Mos *et al.* (1990) compared the effects of different 5-HT_{1A} receptor agonists on sexual behavior in sexually naive and experienced male rats. In the latter group, no statistically significant effects were found with buspirone (0.1-1.0 mg IP). In sexually naive male rats, the mean ejaculation frequency in a 30 min pair-test increased significantly from 0.3 (vehicle) to 1.2 (3 mg buspirone IP). However, after administration of 10 mg buspirone, the mean ejaculation frequency was 0.1. Ipsapirone (3-10 mg IP) reduced significantly the ejaculation latency in sexually naive rats, but didn't have any significant effects in experienced animals. In this study, the number of intromissions prior to ejaculation was not indicated.

HUMAN STUDIES AND 5-HT_{1A} RECEPTOR AGONISTS

The 5-HT_{1A} receptor agonists buspirone, gepirone and ipsapirone are used in clinical practice and clinical trials as a treatment for anxiety disorders and depression. Although to present date no experiments have been carried out to investigate the effects of these drugs on sexual function, there are some indications that they exert some effect on ejaculation. Buspirone may reverse inhibition of ejaculation caused by selective serotonin reuptake inhibitors and improve sexual function in patients with generalized anxiety disorders (Norden, 1994). Crenshaw and Goldberg (1996) report enhanced desire and orgasm, a shorter latency to ejaculation both in men with premature ejaculation and in men with inhibition of ejaculation.

MISCELLANEOUS AGENTS AND SEXUAL BEHAVIOR IN THE MALE RAT

Dopamine

The centrally acting neurotransmitter dopamine is also known for its involvement in control of male rat sexual behavior. Taking the parameters of mount and intromission frequencies and latency to ejaculation as measures of copulatory activity, most reports indicate that dopamine has a stimulatory effect which is exerted via D_2 receptors (Ferrari and Giuliani, 1994). This has been shown extensively in rodents, and also in man by administration of a variety of dopamine receptor agonists followed by D_2 receptor antagonists which reverse their effects (Wilson 1993). Examples of dopamine receptor agonists are L-DOPA, apomorphine, lisuride, bromocryptine, quinelorane. Enhancement of the ejaculatory behavior and the decrease in intromission frequency stimulated some authors to call this altered behavior a rat model for "premature ejaculation" (Napoli-Farris *et al.*, 1984). Examples of dopamine receptor antagonists that have been used to reverse the effects of the agonists include pimozide, haloperidol, metoclopramide, sulpiride, clonazide, spiperidone and cisflupenthixol (Wilson 1993). Some of these antagonists reduced copulatory behavior when given alone, but this may have been due to motor disturbances (Baum and Star, 1980). Since the scope of this thesis is to investigate the effects of serotonergic drugs on sexual behavior in rats and men, the effects of above mentioned dopaminergic agents are not described in detail.

Morphine

Several studies have shown that systemic administration of morphine inhibits male rat sexual behavior (McIntosh *et al.*, 1980, Ågmo and Paredes, 1988). Central administration of two morphinomimetic drugs, ß-endorphin and DALA into a lateral ventricle also inhibited male sexual behavior (Meyerson and Terenius, 1977, Pellegrini Quarantotti *et al.*, 1978). However, in a study of Ågmo and Paredes (1988), a small proportion of male rats reacted differently on a low dose of systemic morphine: there was a decrease of ejaculation latency, and in the number of intromissions prior to ejaculation. These conflicting results indicate that at least there is a role for the enkephalines in the modulation of sexual behavior in the male rat (Ågmo *et al.*, 1994), but more research is required to elucidate more details.

Ecstasy

The amphetamine analog MDMA, better known as the recreational drug ecstasy, is known and feared for its neurotoxic properties. It reduces brain concentrations of serotonin by inhibition of the metabolisation and by long-lasting degeneration of 5-HT nerve terminals, as well as by decreasing the number of 5-HT uptake sites (e.g., Battaglia *et al.*, 1987, Ricaurte *et al.*, 1987). In an experiment with male rats, Dornan and collaborators (1991) found that a chronical administration of MDMA, caused less rats to display mounting behavior, and an increase in ejaculation latency in the responders. These results are conflicting with the above described studies with serotonin receptor agonists and antagonists, because a decrease in central 5-HT would cause an increase in male rats sexual behaviors. Probably, since MDMA has such dramatic effects in the brain, other factors may have played an important role in this experiment.

GABA

The neurotransmitter gamma-aminobutric acid (GABA) occurs in the brain tissue (Rang and Dale, 1987). Two distinct types of GABA receptors are recognized: $GABA_A$ and $GABA_B$ (Simmonds, 1983). There is some evidence that the $GABA_B$ receptor agonists (like baclofen) inhibit sexual behavior in male rats, independently from the effects on motor systems (Ågmo and Paredes, 1985). Efforts to discover a role for $GABA_A$ in the modulation of sexual behavior in the rat have failed so far (e.g., Ågmo and Fernández, 1991).

Yohimbine

The $alpha_2$-adrenoceptor blocking agent yohimbine has been known for its aphrodisiacal properties in rats and humans (Crenshaw and Goldberg, 1996). In male rat studies, it increased mounting behavior (Clark *et al.*, 1984) without the need for physiological levels of serum testosterone (Clark *et al.*, 1985a). When looking at the effects on ejaculation, Clark and collaborators (1985b) found a decrease in ejaculation latency, intercopulatory interval, and post-ejaculatory interval.

Serotonin reuptake inhibitors

The selective serotonin reuptake inhibitors (SSRIs) fluoxetine, fluvoxamine, paroxetine and sertraline and the tricyclic antidepressant clomipramine, also with inhibitory properties of reuptake of serotonin are clinically used as

antidepressants in humans. In recent years, these agents are used in the treatment of premature ejaculation in humans (chapter 7). Behavioral experiments with selective 5-HT reuptake inhibitors on rats have shown inhibition of male rat sexual behavior (Yells *et al.*, 1994), a long-lasting decrease of the ejaculatory response after a single dose of fluoxetine (Rényi, 1986) and increase of intermount-bout intervals, grooming time, ejaculation latency, number of mounts per mount bout, and number of mount bouts prior to ejaculation (Yells *et al.*, 1995). In a recently published study, Mos and coworkers (1997) found no major inhibitory effects of clomipramine, fluvoxamine, fluoxetine, clomipramine or paroxetine on male rat sexual behavior at "non-sedative doses" in sexually naive and experienced male rats.

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Chapter 4

Sex behavior of male and female Wistar rats affected by the 5-HT_{1A} receptor agonist 8-OH-DPAT*

PRESENTLY IT IS GENERALLY ACCEPTED that 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) has stimulating properties on sexual behavior in male rats (Ahlenius *et al.*, 1981, Mendelson and Gorzalka, 1986, Ahlenius and Larsson, 1988). At the time of the first publication, however, it was generally thought that serotonin was inhibitory (Ahlenius *et al.*, 1980). Thus, the stimulatory effects of a 5-HT receptor agonist were quite surprising. Later developments in classifying 5-HT_1 subtype receptors showed that 8-OH-DPAT is a powerful and probably full receptor agonist at the 5-HT_{1A} site (Middlemiss and Fozard, 1983).

Other 5-HT_{1A} receptor agonists share the facilitatory effects on male sexual behavior (buspirone, ipsapirone, flesinoxan) thus suggesting the importance of this receptor for sexual behavior (Glaser *et al.*, 1987, Mathes *et al.*, 1990, Mos *et al.*, 1990). In contrast, the 5-HT_{1B} site is thought to inhibit sexual behavior in rats (Fernández-Guasti *et al.*, 1989). Detailed information on the effects of specific 5-HT_{1B} and 5-HT_{1C} receptor agonists is presently not available. The results with 8-OH-DPAT and other 5-HT_{1A} receptor agonists have stimulated further research into the neuropharmacological mechanisms underlying motivational and ejaculatory processes in sexual behavior (Schnur *et al.*, 1989).

The present experiments were carried out to replicate and elaborate some of the earlier reported findings on masculine sexual behavior, both in male and female rats (Haensel *et al.*, 1990, Schnur *et al.*, 1989). Also the possible stimulatory effects of 8-OH-DPAT on male sexual attractivity for a female partner were studied.

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GENERAL METHOD

Animals and treatment

Albino Wistar rats (HSD, Zeist, Holland) were used, except the males in experiments 2 and 3, which were F1-hybrids of two inbred Wistar strains (RxU). The animals were housed two to three to a cage, of the same sex and treatment. Water and food were available *ad lib*. The day-night cycle was artificially maintained (dark 7:30 AM - 5:30 PM) and temperature ranged from 22 to 24°C. Ovariectomies were carried out under light ether anesthesia via bilateral lumbar incisions. Stimulus females were brought into estrus by injecting 30μg estradiol benzoate (EB) in 0.15 ml olive oil 48 or 24 h prior to testing, followed by 2.5 mg progesterone (P) in 0.1 ml oil 3 h before testing. A fresh solution of 8-OH-DPAT [(±) 8-OH-DPAT·HBr; Research Biochemicals Inc., Natick, MA] was made approximately 1 h before testing: 0.2 or 0.4 mg 8-OH-DPAT dissolved in 0.2 ml saline per kg body weight was administered by SC injection in the neck, 30 min prior to testing. Behavioral tests started about two hours after the onset of the dark cycle and the animals were allowed to adapt to the test cage for 15 minutes.

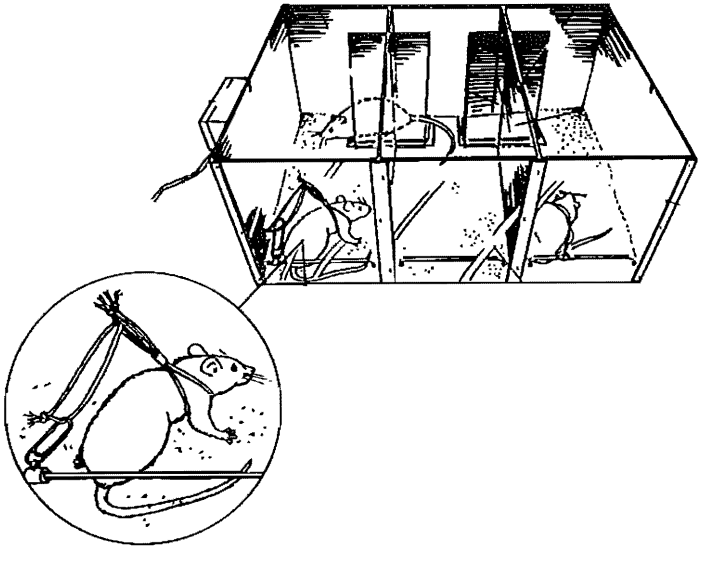

FIGURE 4.1. A modified version of the 3-compartment box used in experiment 4.3 (see text)

Behavioral tests

In pair-tests (experiments 4.1, 4.2 and 4.4) behavioral testing was carried out in a semicircular arena, measuring 62x40x36 cm, with a wire mesh floor and a transparent front (Broere *et al.*, 1985). In the partner preference test (experiment 4.3) a test box made of grey perspex with transparent front was used (figure 4.1; Slob *et al.*, 1987). It consisted of three compartments (60x30x40 cm each) with small openings (13x12 cm) in both partitions near the front window. Each opening could be closed by a sliding door. Stimulus animals were put into the lateral compartments, one male treated with 8-OH-DPAT and one control man with saline. These males were either placed behind a wire mesh separation halfway the lateral compartment (tests 1-4) or given leather harness which was attached with a stainless steel wire to the rear of the compartment to limit their action radius (tests 5-8). They were adapted to the test cage and tethering device twice for 15 minutes in the week before testing. The test room was dimly illuminated with indirect white light (60W).

Statistical analysis

Generally, data were subjected to one- or two-way analyses of variance (ANOVA) for repeated measures (Perlman, 1986). Significant interactions were analysed with the simple main effects method (Kirk, 1968). If the overall test was statistically significant, the Least Significant Difference (LSD) Test was used to make pairwise comparisons among means (Kirk, 1968). Friedman's two-way ANOVA was used for nonparametric analysis (Siegel, 1956). The 0.05 level of probability was adopted as the level of statistical significance.

EXPERIMENT 4.1 - DOES 8-OH-DPAT STIMULATE SEXUAL BEHAVIOR IN MIDDLE-AGED MALE RATS?

It has been shown that 8-OH-DPAT increases ejaculatory performance in male rats (Ahlenius *et al.*, 1981, Arvidsson *et al.*, 1981, Mendelson and Gorzalka, 1986, Schnur *et al.*, 1989). Such studies employed males that were approximately 6 months old, i.e. relatively young. It was thought to be of interest to investigate the stimulatory properties of the drug in middle-aged male rats, particularly because such rats are assumed to have impaired reproductive capabilities and

are generally assumed to be less sexually active (Larsson, 1958, Larsson and Essberg, 1962, Gray *et al.*, 1981).

Method

Twelve middle-aged rats (12-14 months old at the start of the experiment; body weights between 540 and 695 g) were tested for masculine sexual behavior. These animals were former breeding males but had not had sexual experience for at least four months prior to testing. Following the first 15 min pair-test two groups were formed on the basis of their sexual performance: an "active group" (n=7), consisting of males that ejaculated at least once and an "inactive group" (n=5) of males that did not ejaculate.

The animals were tested once a week with an estrous female for 7 consecutive weeks. When a stable level of sexual behavior was found (i.e. tests 3 and 4), treatment with 8-OH-DPAT or saline was started. In tests 5, 6 and 7 the animals were treated with saline (2 ml/kg), 8-OH-DPAT (0.2 mg/kg) and saline (2 ml/kg), respectively. Finally, test 8 (saline) was given 4 weeks following test 7.

TABLE 4.1. Sexual behavior parameters (means ± SE) in sexually active (A, n=7) and inactive (I, n=5) middle-aged male Wistar rats

		WEEKLY TESTS RELATIVE TO 0.2 mg/kg 8-OH-DPAT TREATMENT					Statistics (two-way ANOVA) on last 4 tests; F-values[2]
Behavior		-5 (n)[1]	-1 (n)	8-OH-DPAT	1 (n)	5 (n)	
Mounts							
number	A	12.1 ± 2.3 (7)	15.0 ± 3.9 (7)	7.3 ± 2.3 (7)	13.7 ± 3.8 (7)	10.4 ± 2.7 (7)	t: F= 4.3, p<0.02
	I	11.6 ± 6.4 (2)	18.0 ± 2.2 (5)	8.8 ± 1.9 (5)	13.4 ± 2.6 (5)	22.2 ± 4.7 (5)	LSD(5%)=4.3
Intromissions							
number	A	13.0 ± 1.1 (7)	13.3 ± 1.4 (7)	9.0 ± 1.0 (7)	10.6 ± 1.7 (7)	12.9 ± 1.0 (7)	gr x t: F= 3.1, p= 0.04
	I	2.4 ± 1.4 (2)	10.8 ± 2.3 (5)	9.2 ± 0.8 (5)	13.2 ± 2.0 (5)	7.6 ± 1.5 (5)	
prior to 1st ejac	A	8.4 ± 1.0	10.1 ± 1.3	4.4 ± 0.9	7.3 ± 2.4	8.7 ± 0.5	[3]
	I	-	12.0 ± 0.7	3.9 ± 1.2	8.7 ± 1.0	9.7 ± 1.1	
Ejaculations	A	1.7 ± 0.2 (7)	1.6 ± 0.2 (7)	2.6 ± 0.3 (7)	1.4 ± 0.4 (6)	1.4 ± 0.4 (6)	t: F= 8.8, p<0.001
number	I	(0)	0.6 ± 0.4 (2)	2.2 ± 0.2 (5)	1.0 ± 0.5 (3)	0.6 ± 0.2 (3)	LSD(5%)=0.6
							gr: F(=4.2, p<0.07
Latencies (sec)[4]							
ML	A	20.0 ± 5.5	7.3 ± 1.0	7.1 ± 1.1	8.1 ± 1.6	9.1 ± 1.4	gr: F= 3, p= 0.096
	I	578.0 ± 281.0	27.2 ± 12.0	10.2 ± 1.9	27.8 ± 15.0	12.7 ± 2.4	
EL	A	360 ± 37	393 ± 40	393 ± 40	511 ± 111	520 ± 109	test: F= 9, p<0.001
	I	900	763 ± 113	763 ± 101	595 ± 156	673 ± 96	LSD(5%)=246

Weekly pair-tests (15 min) with estrous female
[1] Number of subjects displaying the behavior
[2] 2-way ANOVA, factors tests (t; F(3,30)-values indicated), groups (gr; F(1,10)-values)
[3] Responders only; data not suitable for ANOVA, see also experiment 4.2
[4] 900 sec for non responders

Results

Ejaculation behavior is shown in figure 4.2. During 8-OH-DPAT treatment there was a clear stimulation of the mean number of ejaculations in both groups of males. Statistical analysis (two-way ANOVA on tests -1 to +5) revealed an effect of tests, $F(3,30)=8.752$, $p<0.001$; $LSD(5\%)=0.61$, a borderline significant effect of groups, $F(1,10)=4.177$, $p<0.07$, and no significant interaction, $F(3,30)=0.47$, n.s. From inspection of the results it is clear that during 8-OH-DPAT treatment all males of both groups ejaculated with high frequencies. During 8-OH-DPAT treatment males of both groups did not differ in ejaculation frequencies, which were in both groups significantly higher than during the tests before and after.

Various other sexual behavior parameters are presented in table 4.1. With regard to mounting behavior it was found that for both groups of males lowest frequencies were observed during tests with 8-OH-DPAT treatment (two-way ANOVA on tests -1 to +5, groups, $F(1,10)=1.01$, n.s.; tests, $F(3,30)=4.31$, $p<0.02$; groups x tests interaction, $F(3,30)=2.16$, n.s.; $LSD(5\%;tests)=4.32$). For intromission frequencies similar results were obtained, i.e. lowest frequencies with 8-OH-DPAT treatment (two-way ANOVA, groups $F(1,10)=0.62$, n.s.; tests $F(3,30)=2.10$, n.s.; groups x test interaction $F(3,30)=3.08$, $p=0.04$). It is clear that the number of intromissions to first ejaculation did not seem to differ between the two groups of males; lowest numbers were found during 8-OH-DPAT treatment (see also experiment 4.2).

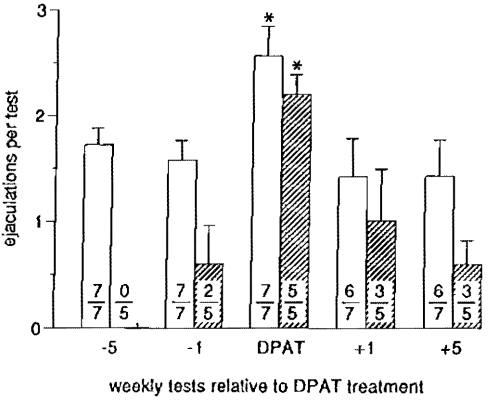

FIGURE 4.2. Experiment 4.1. Mean (± SE) ejaculation frequency of initially active (n= 7; open bars) and inactive (n= 5; hatched bars) middle aged male rats before and after 0.2 mg/kg 8-OH-DPAT treatment. Males were tested weekly for 7 consecutive weeks. The results of the 1st (-5), 5th through 7th (-1, 8-OH-DPAT, +1) and the 8th (+5) test (4 weeks following test 7) are depicted. Asteriks indicate a significant difference from tests before and after treatment (see also table 4.1). Also indicated is the number of animals per test that ejaculated at least once

Two-way ANOVA of mount latencies (test -1 through +5) did not reveal significant differences (groups, $F(1,10)=3.38$, $p=0.096$; tests, $F(3,30)=0.89$, n.s.; groups x tests interaction, $F(3,30)=1.33$, n.s.). Latency to first ejaculation differed significantly over the tests ($F(3,30)=9.46$, $p<0.001$, $LSD(1\%)= 246.0$), but the

groups of males did not differ, F(1,10)=2.65, n.s., and there was also no significant interaction, F(3,30)=1.02, n.s.

Thus there was a trend for overall group difference for latency to first mount, while the males did not differ for first ejaculation latency. Both groups showed shortest latencies to ejaculation with 8-OH-DPAT treatment.

EXPERIMENT 4.2 - "PREMATURE EJACULATION" CAUSED BY 8-OH-DPAT

It has been noted that with 8-OH-DPAT male rats can ejaculate already at the first intromission (e.g., Ahlenius *et al.*, 1981). This phenomenon, which may be called "premature ejaculation" by analogy with what is described in the human (e.g., Masters and Johnson, 1970), was investigated in more detail. Questions that were studied comprised firstly the quality of the ejaculate, whether or not it contained mobile spermatozoa, secondly where the semen was deposited, i.e. intra- or extravaginally and thirdly the details of the sexual behavior.

Method

Twenty heterosexually naive male rats, approximately 6 months old, were pair-tested for sexual behavior. Tests lasted till one ejaculation had occurred. The numbers of mounts and intromissions prior to ejaculation were scored, and latencies to first mount (ML), first intromission (IL) and ejaculation (EL) were calculated. Immediately after ejaculation of the male the stimulus female was removed from the test cage and a vaginal smear was taken, which was subsequently studied microscopically. The test cage was carefully searched for the presence of a seminal plug; both animals were also thoroughly inspected for possible ejaculate coagulated to their furs.

Five consecutive weekly tests were carried out in which the animals received saline (2 ml/kg), 0.2 mg/kg 8-OH-DPAT, 0.4 mg/kg 8-OH-DPAT, saline (2 ml/kg), and 0.4 mg/kg 8-OH-DPAT, respectively.

Results

Various sexual behavior parameters are presented in table 4.2. The main findings are that latency to ejaculation is significantly shorter with 8-OH-DPAT treatment. Mean number of intromissions prior to ejaculation and latency to first ejaculation decreased significantly with 8-OH-DPAT treatment. Although lowest

values were found with the higher dose, this was not significantly different from the lower dose 8-OH-DPAT. "Premature ejaculations" (i.e., ejaculations at the first or second intromission) were never observed following saline injection (tests 1 and 4). With the low dose of 8-OH-DPAT (tests 3 and 5), 11 and 10 males, respectively, ejaculated during their first or second intromission.

TABLE 4.2. Experiment 4.2. Sexual behavior parameters (means ± SE) in male rats (n=20) tested until first ejaculation

Behavior	Test 1 saline	Test 2 8-OH-DPAT 0.2 mg/kg	Test 3 8-OH-DPAT 0.4 mg/kg	Test 4 saline	Test 5 8-OH-DPAT 0.4 mg/kg	Statistics (one-way ANOVA) F(4,76)-value
Intromissions:						
number	12.7 ± 0.6	2.4 ± 0.6	1.6 ± 0.4	14.0 ± 1.1	1.8 ± 0.3	F=7.54, p<0.001 LSD(5%)= 1.4
range	8-18	0-14	0-5	6-26	0-4	
Latency to 1st intromission (s)	76.1 ± 24.7	28.9 ± 10.4	6.8 ± 1.3	14.7 ± 2.2	12.2 ± 4.4	F=6.05, p<0.001 LSD(5%)= 27.0
Ejaculation:						
latency	505 ± 52	123 ± 47	36 ± 13	289 ± 28	39 ± 9	F=37.0, p<0.001 LSD(5%)= 7.4
1st intro-ejac[1]	0	1	7	0	2	
2nd intro-ejac[1]	0	8	4	0	8	
Ejaculatory plug:						
intravaginal	16	15	10	19	14	
extravaginal	1	1	7	1	5	
combined	2	1	2	0	0	
not found	1	3	1	0	1	

Five weekly tests, with 8-OH-DPAT or saline treatment prior to testing
[1] Number of animals ejaculating at the first or second intromission

With regard to the location of the semen it was found that this was usually the vagina. However, with the higher dose 8-OH-DPAT (tests 3 and 5) 7 and 5 animals, respectively, were found to ejaculate extravaginally. Surprisingly, this was also observed in one male in test 1 and one other male in test 4. The quality of the ejaculate, as judged by microscopic inspection revealed no systematic differences between the tests. Live spermatozoa were always found.

EXPERIMENT 4.3 - DOES 8-OH-DPAT RENDER MALES MORE SEXUALLY ATTRACTIVE TO FEMALES?

When given the choice in a 3-compartment box between 2 tethered stimulus males, one intact and one castrated, an estrous female rat prefers the vicinity of

the castrated male (Broekman *et al.*, 1988, De Bruijn *et al.*, 1988). Females' preference for the castrated rat was mainly due to the aversion to genital stimulation received during intromissions by the intact male. When intromissions were prevented through vaginal occlusion, intact males became by far the preferred partners (Broekman *et al.*, 1988). 8-OH-DPAT treatment was reported to decrease mounting and intromission frequencies in male rats (Ahlenius *et al.*, 1981, Ahlenius and Larsson, 1988, Schnur *et al.*, 1989; see also experiments 4.1 and 4.2). Therefore, it was postulated that 8-OH-DPAT treatment could make males more attractive as a mating partner for an estrous female rat. This idea was tested in the following experiment.

Method

Fifteen heterosexually naive female rats were tested for partner preference behavior in a 3-compartment box (figure 4.1). They were ovariectomized at the age of 3 months and behavioral testing started one month later. They were brought into behavioral estrus by EB, 20 μg 48 and 24 h prior to testing, followed by P, 2.5 mg 3 h before the 15-min test. The stimulus males (n=30) were treated with saline or 8-OH-DPAT (0.2 mg/kg) 30 min prior to testing and put in the lateral compartments of the 3-compartment box. At the start of the test the sliding doors were removed and the time spent in each compartment was recorded. To quantify the partner preference, a preference score was calculated in which the time spent with the saline-treated male was subtracted from the time spent with the 8-OH-DPAT male. Thus, a positive score indicates a preference for the 8-OH-DPAT male, while a negative score indicates means that the control male is preferred. In tests 1-4 the males were kept behind wire mesh, whereas in tests 5-8 this wire mesh partition was removed and behavioral interaction was possible between the estrous female and the tethered males. During the latter 4 tests various sexual behavior data were recorded.

Results

Partner preference behavior. The data of tests 1-8 were subjected to one-way ANOVA. There appeared to be no significant preference for either partner, $F(7,98)=1.28$, n.s.; see figure 4.3. The time spent in the empty middle compartment revealed interesting results. One-way ANOVA (tests 1-8) showed a significant effect, $F(7,98)=14.43$, $p<0.001$. Further LSD analysis [LSD(5%)=69.9;

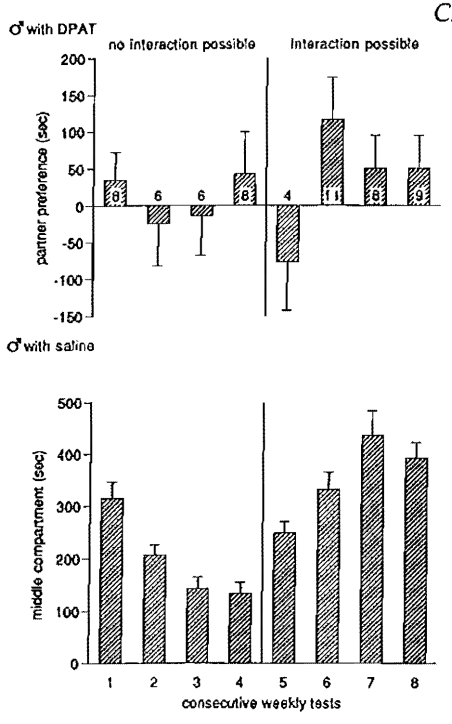

FIGURE 4.3. Experiment 4.3. (Top) Mean (± SE) preference (sec) for a 0.2 mg/kg 8-OH-DPAT-treated male over a saline-treated male of estrous female rats (n=15), tested in a 3-compartment box (3CB; 15 min/test). During tests 1-4 behavioral interaction was prevented by a wire mesh partition, which was removed during tests 5-8. Figures in bars indicate number of females that preferred the vicinity of the 8-OH-DPAT-treated male (i.e. a positive time score). (Bottom) Mean (±SE) time (sec) spent in the empty middle compartment of the 3CB. From this also the total time in the lateral compartments may be calculated: total test time adds up to 900 sec. For example, test 4: 134 sec in middle compartment means 766 sec (900 - 134) in lateral compartments.

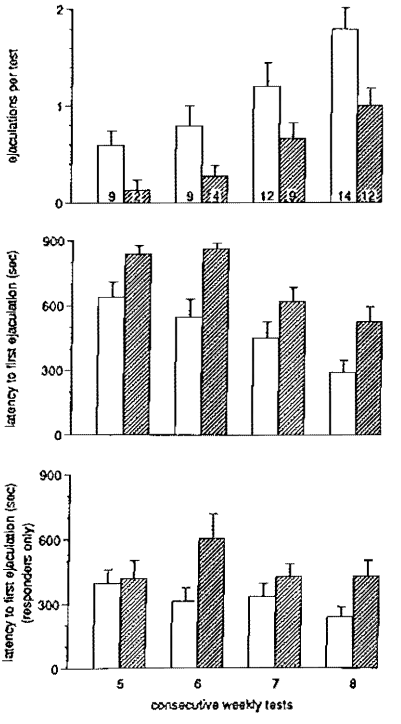

FIGURE 4.4. Experiment 4.3. Various ejaculatory parameters (mean ± SE) of the 8-OH-DPAT-treated (n=15, open bars) and saline-treated (n=15, hatched bars) tethered males during partner preference test 5-8 as depicted in figure 4.3. Figures in bars indicate number of males that ejaculated at least once during that test.

LSD(1%)=99.3] indicated that during tests 3 and 4 (no behavioral interaction with stimulus males possible) the estrous females spent most of the time in the lateral compartments which contained the males. In tests 7 and 8 the estrous females spent about half of the total time middle compartment although in the remaining time they were sexually very active with the tethered males (see later, figures 4.4 and 4.5).

Sexual behavior with tethered males. Various sexual behavioral parameters are presented in figures 4.4 and 4.5. Two-way ANOVA of ejaculation frequencies

(figure 4.4, top) revealed a significant effect of tests, $F(3,42)=19.39$, $p<0.001$, of treatment of the stimulus males, $F(1,14)=19.27$, $p<0.001$, but no significant interaction, $F(3,42)=0.37$, n.s. Thus the females received a progressively increasing number of ejaculations over the 4 tests, while overall the 8-OH-DPAT treated males were 'preferred' in this respect. Only during test 5 (the first test with behavioral interaction possible), there was a significant difference between the number of 8-OH-DPAT- and saline-treated males which ejaculated 9/15 vs. 2/15, respectively (Fisher exact probability test $p=5.17$, $df=1$, $p<0.02$).

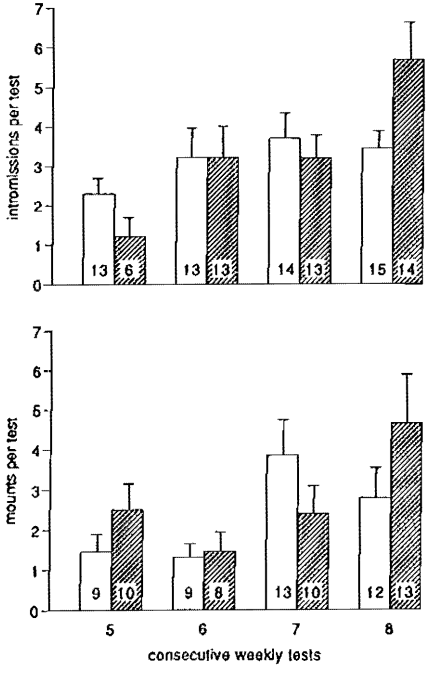

FIGURE 4.5. Experiment 4.3. Intromission (top) and mount (bottom) (means ± SE) of the 8-OH-DPAT-treated (n=15, open bars) and saline-treated (n=15, hatched bars) tethered males during partner preference test 5-8 as depicted in figure 4.3. Figures in bars indicate number of males that showed the behavior.

The mean latencies to first ejaculation (figure 4.3, middle; with 900 s cut-off value for nonejaculators), subjected to Friedman's two-way ANOVA, showed a significant effect of tests, $X_r^2(3)=15.12$, $p<0.001$, and treatment, $X_r^2(1)=19.27$, $p<0.001$, but no significant interaction was apparent. Hence, over the 4 tests, females received the first ejaculation progressively sooner after the beginning of testing, while the shortest latencies occurred with the 8-OH-DPAT-treated males.

Mean latencies to first ejaculation of responders only (figure 4.4, bottom) were significantly different between 8-OH-DPAT- and saline-treated males in tests 6, $t(11)=2.31$, $p<0.05$, and 8, $t(24)=2.28$, $p<0.05$.

Intromission frequencies (figure 4.5), subjected to two-way ANOVA, differed over the tests, $F(3,42)=5.67$, $p=0.002$, with no overall treatment difference, $F(1,14)=0.21$, n.s., but with a significant test x treatment interaction, $F(3,42)=4.66$, $p=0.007$. This interaction points to the fact that the 8-OH-DPAT-treated males (figure 4.5, open bars) had somewhat similar mean intromission frequencies over the 4 tests (range: 2.3-3.7), while the saline-treated males (hatched bars) showed

an increase over the tests (range: 1.2-5.7). Mount frequencies subjected to ANOVA showed a significant effect of tests, $F(3,42)=5.17$, $p<0.004$, no effect of treatment, $F(1,14)=0.41$, n.s., and a test x treatment interaction, $F(3,42)=2.75$, $p=0.05$.

In summary, in the course of testing there is an overall increase in copulatory activity of the females with the tethered males. With regard to the number of ejaculations and latency to first intromissions, the 8-OH-DPAT males are more often preferred and are allowed to be more sexually active.

EXPERIMENT 4.4 - DOES 8-OH-DPAT STIMULATE MOUNTING BEHAVIOR IN FEMALE RATS?

8-OH-DPAT has stimulating properties in male rats for sexual behavior, and more specifically ejaculation behavior (Ahlenius *et al.*, 1980, Schnur *et al.*, 1989; see also experiment 4.2). Female rats with and sometimes without exogenous testosterone readily mount an estrous conspecific (e.g., Beach, 1968, Brand *et al.*, 1990). The question was investigated whether or not 8-OH-DPAT also had "aphrodisiac" properties in female rats.

Method

Seventeen heterosexually naive female rats, approximately 3 months old, were ovariectomized. Three weeks later behavioral testing started, i.e., a 15 min pair-test with an estrous female. Testing occurred twice weekly, 3 or 4 days apart. Animals were tested in three series of 7 tests each. In each test series these 7 tests comprised a first and a last test without any exogenous treatment and tests 2 through 6 with SC injections with saline, 8-OH-DPAT (0.2 mg/kg), saline, 8-OH-DPAT (0.4 mg/kg) and saline, respectively.

Following the first test series each female received SC in the neck under light ether anesthesia a silastic implant filled with testosterone (inner diameter 1.5 mm, outer diameter 2.1 mm, effective length 2 cm). Three weeks later test series 2 was started. One week following the last test of the second test series the implants were removed and the females were left undisturbed for 7 weeks. Then the final test series were carried out.

During behavioral tests mounts with pelvic thrusts and intromission-like mounts were scored, and latency to first mount was recorded.

Results

Mount frequencies and latencies to first mount are delineated in figure 4.6. The data were subjected to two-way ANOVA and were normalized by taking the square root of each of the data (Kirk, 1968) with factors testosterone and saline/8-OH-DPAT treatment. For mount frequencies there was an effect of testosterone, $F(2,320)= 152.5$, $p<0.001$, of saline/8-OH-DPAT treatment, $F(6,320)= 2.70$, $p<0.01$, and a significant interaction, $F(2,320)= 3.12$, $p<0.01$. Analysis with the simple main effects method (15) only showed a significant overall effect during testosterone treatment, $F(6,320)= 5.85$, $p<0.001$; test series 1 and 3 did not differ, $F(6,320)= 1.60$, n.s. and $F(6,320)= 1.50$, n.s., respectively. Subsequent analysis with LSD method (LSD(5%)= 1.59 for transformed data) revealed that the highest levels of mounting behavior were displayed during 8-OH-DPAT treatment. Transformed (square root) data for latency to first mount subjected to two-way ANOVA, only showed a significant effect of testosterone, $F(2,32)= 35.45$, $p<0.001$, no effect of tests, $F(6,96)= 0.83$, n.s. and no significant interaction, $F(12,192)= 1.69$, $p=0.07$. LSD analysis of the data during testosterone [LSD(5%)=4.82 for transformed data] showed shortest latencies to mount with 8-OH-DPAT treatment.

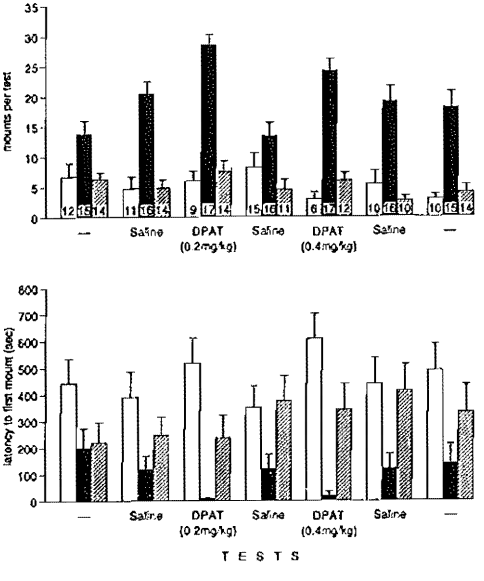

FIGURE 4.6. Experiment 4.4. Mounts and latency to first mount (mean ± SE) of female rats (n=17) during 3 test series: before (open bars), during (black bars) and after (hatched bars) long-term testosterone treatment. Each test-series comprised 7 tests (15 min/test, twice weekly) in a fixed sequence of treatment: blank, saline, 0.2 mg/kg 8-OH-DPAT, saline, 0.4 mg/kg 8-OH-DPAT, saline and blank.

Occasionally females displayed intromission-like behaviors. Very low frequencies were seen in tests without 8-OH-DPAT in all 3 test series (range 0-3 "intromissions" in 17 females). 8-OH-DPAT treatment increased the number of females displaying this behavior: low dose 8-OH-DPAT 3-5 out of 17; the higher dose 8-OH-DPAT 6-11 out of 17. The highest frequency, 11 of 17 females, was observed in the testosterone condition (i.e., test series 2).

GENERAL DISCUSSION

The main findings of the present experiments can be summarized as follows. 8-OH-DPAT treatment stimulated ejaculation frequency in "middle-aged" (approximately 15 months old) male rats, both in initially sexually active and in initially sexually inactive subjects. 8-OH-DPAT made both groups equally sexually active. The increased number of ejaculations per test coincided with a decrease in total number of mounts, of intromissions, of intromissions prior to first ejaculation and of latency to first ejaculation. In the second experiment 8-OH-DPAT rendered a high percentage (45-55%) of males to ejaculate "prematurely," i.e., at the first or second intromission. Latency to ejaculation decreased. With the higher dose 8-OH-DPAT (0.4 mg/kg) 25-35% of the males ejaculated extravaginally.

8-OH-DPAT treatment did not make males more attractive for an estrous female, as judged by the time spent in the neighborhood of such males. The estrous females received far more ejaculations from the tethered 8-OH-DPAT-treated males, with the lowest latencies to first ejaculation, than from saline-treated males.

In the final experiment it was found that 8-OH-DPAT treatment stimulated mounting behavior in female rats only when they were long-term treated with testosterone. During testosterone treatment the shortest latencies to first mount were found after 8-OH-DPAT administration.

The facilitatory effects of 8-OH-DPAT on male sexual behavior and sexual motivation as judged by latency to ejaculation in our middle-aged male rats are similar to what has been found earlier in younger rats (Middlemiss and Fozard, 1983, Ahlenius *et al.*, 1981, Schnur *et al.*, 1989). The stimulatory effects of 8-OH-DPAT in sexually active and inactive middle-aged gonadally intact male rats seem to be a new finding.

The premature character of the ejaculatory behavior following 8-OH-DPAT treatment is in line with other studies (Ahlenius *et al.*, 1981, Mendelson and Gorzalka, 1986). Not reported in the literature is the finding that relatively many males (25-35%) ejaculated extravaginally. Two other studies that collected and examined the coagulated ejaculate or copulatory plugs reported an "impairment in ejaculation" (Schnur *et al.*, 1989) or "a trend towards weight reduction" (Lee *et al.*, 1990). These conclusions were based on lower plug weights with 8-OH-DPAT.

The mechanism through which the 5-HT_{1A} receptor agonist 8-OH-DPAT facilitates various aspects of ejaculatory behavior is not yet clear. It could potentially affect animals via both central and peripheral pathways. Finberg and Vardi (1990), studying the inhibitory effects of 5-HT on penile erectile function in the pithed rat, found that 8-OH-DPAT had no effect on intracavernous corporal pressure in the penis. This suggests that 8-OH-DPAT does exert its effects centrally rather than peripherally. The present results with the testosterone-treated females (experiment 4.4) also point towards a central action of the drug.

Although 8-OH-DPAT treatment stimulates sexual behavior in males, it does not make them more attractive to estrous female conspecifics. When given the choice between 8-OH-DPAT-treated and saline-treated males, females have no preference for either one as judged by the time spent in their vicinity. It does not make a difference whether or not sexual behavior with the stimulus males is possible. When sexual behavior is possible, females copulated with both males but spent most time in the neutral compartment. This contrasted with the situation in which sexual interaction was prevented with a wire mesh partition. Then, females spent most of their time in proximity of either male. It was earlier found that estrous females prefer a castrated male over a gonadally intact male (Broekman *et al.*, 1988, De Bruijn *et al.*, 1988). Such preference disappeared when the vagina was taped, which prevented intromissions and ejaculations to occur (Broekman *et al.*, 1988). Although in the present experiment 8-OH-DPAT-treated males had lower intromission frequencies to ejaculation than controls this did not make them more attractive. This can be explained by the fact that the total intromittive activity was virtually similar for both stimulus males. In a recently published paper flesinoxan, just as 8-OH-DPAT a 5-HT_{1A} receptor agonist, increased ejaculation frequencies in tethered male rats and made them more attractive to estrous females (Mos *et al.*, 1990). This latter finding differs from the present results. There are many differences in the testing procedures between the two studies. For instance, Mos *et al.* (1990) administered different drugs (a total of 5) or vehicle in a cross-over design to each of 8 pair of male rats, with intervals between the tests of 3 to 4 days. It remains to be tested whether flesinoxan renders tethered males more attractive when animals are tested repeatedly as was done in the present study.

In the present study, the facilitatory effects of 8-OH-DPAT on mounting behavior in female rats were only present with concomitant long-term testosterone treatment. Also 8-OH-DPAT enhanced sexual motivation in

testosterone-treated female rats, as judged by latency to first mount. These data corroborate the preliminary results of Mendelson and Gorzalka (1986). In the latter study females were testosterone treated for 3 weeks (SC 100 μg TP/day) and tested only once for masculine sex behavior. Although in the present study it was investigated in much more detail (repeated twice weekly tests, before, during and after testosterone treatment), basically the same results were obtained.

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SUMMARY

Four experiments were carried out to test the stimulatory effects of 8-OH-DPAT on various aspects of "masculine" sexual behavior of male and female rats and on the sexual attractivity of male rats. In experiment 4.1 8-OH-DPAT (0.2 mg/kg) stimulated ejaculation frequency in middle-aged (approximately 15 months old) males, both sexually inactive and active subjects. There was a coinciding decrease in total numbers of mounts, intromissions, intromissions prior to first ejaculation and latency to first ejaculation. In experiment 4.2 the effects of the two doses (0.2 and 0.4 mg/kg) 8-OH-DPAT on the first ejaculation cycle were investigated. Especially, the higher dose made a high percentage (45-55%) of males to ejaculate "prematurely," i.e. at the first or second intromission. Latency to ejaculation decreased. With the higher dose, 25-35% of the males ejaculated extravaginally. In experiment 4.3, 8-OH-DPAT did not make males more attractive for an estrous female than saline-treated males, as judged by time spent in their vicinity. However, estrous females received much more ejaculations from the tethered 8-OH-DPAT males, with the lowest latencies to first ejaculation, than from the saline-treated males. In experiment 4.4 8-OH-DPAT stimulated mounting behavior in female rats only when they were long-

term treated with testosterone. In that condition also shortest latencies to first mount were found with 8-OH-DPAT treatment.

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Chapter 5

Testosterone is required for the stimulatory effects of 8-OH-DPAT on sexual behavior in castrated male rats*

THE EFFECTS OF 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on male and to a lesser extent on female sexual behavior have been studied by several groups for more than a decade (Ahlenius and Larsson, 1991). Recently, we reported on the effects of 8-OH-DPAT on the sexual behavior of male and female rats (chapter 4). In female rats, it was found that 8-OH-DPAT had stimulatory properties on mounting behavior, i.e. increase in frequency, when the animals had received a long-term treatment with testosterone. In the absence of testosterone 8-OH-DPAT had no effects on female mounting behavior. It was concluded that the effect of 8-OH-DPAT on mounting behavior was dependent on the presence of testosterone. This conclusion is at variance with that drawn from studies with male rats. Ahlenius *et al.* (1981) observed a significant stimulation of male rat sexual behavior (increase in numbers of mounts and intromissions) at about 5 weeks after castration and suggested that 8-OH-DPAT did not require testosterone to stimulate sexual behavior. The present experiment was conducted to investigate whether testosterone was required for 8-OH-DPAT to affect the copulatory behavior of castrated male rats.

METHOD

Animals and laboratory conditions

Twelve heterosexually experienced male rats, locally bred F_1-hybrids of two inbred Wistar strains (RxU) were used. At the start of study, they were 10

* Haensel SM, J Mos, P van der Schoot, AK Slob. Testosterone is required for the stimulatory effects of 8-OH-DPAT on sexual behavior in castrated male rats. *Eur J Pharmacol* 1993, 233: 187-192

months old and weighed between 400-500 g (mean 460 g). Female stimulus animals were Wistar rats (HSD, Zeist, The Netherlands) of approximately the same age as the male rats. The animals were housed two or three to a cage. Water and food were available *ad lib.* The light-dark cycle was artificially maintained (dark between 7:30 AM and 5:30 PM) and the temperature ranged from 20 to 22°C in the air-conditioned room. The rats were kept under the same conditions throughout the experiment.

Operations and drugs

Stimulus females were ovariectomized via bilateral incisions under light ether anesthesia. They were brought into behavioral estrus once a week by injecting 30 μg estradiol benzoate in 0.15 ml olive oil 48 or 24 h before testing, followed by 2.5 mg progesterone in 0.1 ml olive oil 3 h before testing. Male rats were castrated through a single incision in the ventral abdominal wall under ether anesthesia.

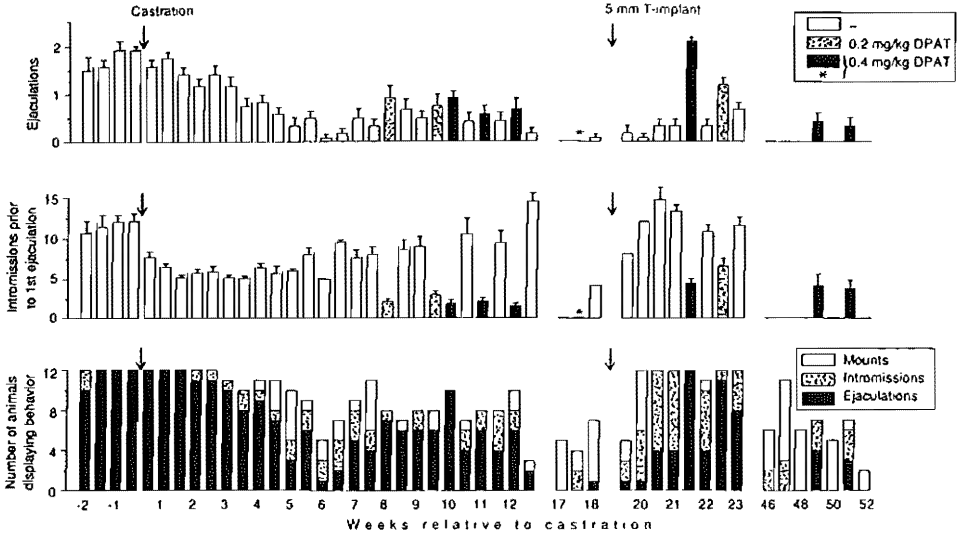

FIGURE 5.1. Three parameters of sexual behavior of male Wistar rats (n=12) before and after castration. Animals were tested with an estrous female (15 min, twice weekly up to week 23 after castration; once weekly last 7 weeks). The behavioral effects of 0.2 and 0.4 mg/kg 8-OH-DPAT were studied. A 5-mm Silastic testosterone-filled tube, implanted in week 19, resulted in subnormal serum testosterone levels, ranging from 4.6 nmol/l 20 weeks after castration to 0.6 nmol/l after 52 weeks. From week 7 onwards animals were injected with saline or 8-OH-DPAT 30 min before testing.

A fresh solution of 8-OH-DPAT ((±)-8-hydroxy-2-di-n-propylamino) tetralin·HBr; Research Biochemicals Inc., Natick, MA, USA) was made approximately 1 h before testing: 0.2 or 0.4 mg 8-OH-DPAT dissolved in 0.2 ml saline per kg body weight was administered by SC injection in the neck, 30 min before testing. From 7 weeks after castration onwards, the animals were always

TABLE 5.1. Effects of castration on sexual behavior (means ± SE) of 12 male rats. Castration was performed after the last test in block 1.

Behaviour	Block 1 (week-2 to 1)	Block 2 (week 1-2)	Block 3 (week 3-4)	Block 4 (Week 5-6)	F(3,33)	p	LSD (5%)	LSD (1%)
Mts	10.4 ± 0.8	13.2 ± 2.0	10.0 ± 1.3	7.7 ± 1.1	3.1	< 0.04	3.0	4.4
Int	15.8 ± 0.6	8.2 ± 0.3	5.6 ± 0.5	3.0 ± 0.5	163.6	<0.001	1.0	1.5
Ejac	1.8 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	51.6	<0.001	0.2	0.3
ML	4.5 ± 0.6	7.1 ± 2.1	98.5 ± 34.4	276.5 ± 77.1	10.4	<0.001	95	137
IL	17.8 ± 3.5	38.8 ± 9.6	202.6 ± 54.3	494.0 ± 65.3	32.6	<0.001	93	134
EL	389.3 ± 37.3	291.3 ± 27.4	421.4 ± 57.9	702.8 ± 47.4	24.7	<0.001	85	123
Before 1st ejaculation								
Mts	7.0 ± 0.9	9.5 ± 1.4	8.9 ± 1.4	11.1 ± 1.4	2.7	(0.06)	-	-
Int	11.7 ± 0.7	6.4 ± 0.3	5.6 ± 0.3	7.2 ± 0.5	41.3	<0.001	1.0	-
Ejaculators only								
ML	4.5 ± 0.6	7.1 ± 2.1	25.2 ± 6.1	52.2 ± 20.5	4.6	<0.01	25	36
IL	17.8 ± 3.4	36.8 ± 9.6	69.8 ± 19.2	66.3 ± 21.1	2.4	(0.08)	-	-
EL	362.3 ± 40.2	276.0 ± 28.8	310.5 ± 40.9	374.8 ± 47.9	4.1	<0.02	55	-

Animals were tested (15 min) twice weekly. Block data were subjected to a one-way ANOVA.
[1] Various sexual behaviors were analysed: mounts (Mts), intromissions (Int), ejaculations (Ejac), and latencies (sec) to first mount (ML), to first intromission (IL) and to first ejaculation (EL)

injected with saline or 8-OH-DPAT 30 min before being tested with estrous female rats.

For testosterone treatment a hormone filled silastic capsule (Talas, Ommen, The Netherlands; inner diameter 1.5 mm, outer diameter 2.1 mm, length 5 mm) was implanted SC in the neck.

Blood collection and hormone assay

Blood was collected from the orbital plexus under light ether anesthesia 19, 23, 46 and 52 weeks after castration. Testosterone concentrations were estimated in serum by radio immunoassay, without chromatography, using the method currently used in our laboratory (Verjans *et al.*, 1973). The intraassay and interassay coefficients of variation were 3 and 5%, respectively.

TABLE 5.2. Effects of two doses of 8-OH-DPAT on sexual behavior (means ± SE) of 12 male rats castrated 8-12 weeks before testing.

Behavior[1]	saline[2]	8-OH-DPAT 0.2 mg/kg[3]	F(1,11)	p	saline[4]	8-OH-DPAT 0.4 mg/kg[5]	F(1,11)	p
Mts	8.3 ± 2.3	3.8 ± 1.1	3.8	(0.08)	6.1 ± 2.0	2.9 ± 0.9	2.2	n.s.
Int	4.7 ± 1.2	2.2 ± 0.6	3.6	(0.08)	3.4 ± 1.0	1.6 ± 0.3	2.6	n.s.
Ejac	0.4 ± 0.1	0.8 ± 0.2	2.3	n.s.	0.3 ± 0.1	0.7 ± 0.1	3.8	(0.08)
ML	220.5 ± 85.6	342.7 ± 123.7	0.6	n.s.	454.5 ± 68.1	212.8 ± 99.0	2.9	n.s.
IL	407.6 ± 114.7	433.1 ± 123.1	0.0	n.s.	611.7 ± 72.4	298.6 ± 97.4	6.0	<0.04
EL	699.8 ± 73.7	474.5 ± 113.9	2.6	n.s.	762.8 ± 51.9	436.8 ± 80.5	10.6	<0.01
Before 1st ejaculation			F(1,3)				F(1,5)	
Mts	15.0 ± 6.1	3.2 ± 1.2	4.1	n.s.	12.9 ± 5.2	3.0 ± 1.4	3.1	n.s.
Int	9.1 ± 1.1	2.5 ± 0.4	49.3	<0.01	10.2 ± 1.4	1.7 ± 0.6	29.3	<0.01
Ejaculators only								
ML	6.8 ± 0.8	10.6 ± 3.7	0.8	n.s.	9.2 ± 1.2	18.9 ± 10.7	0.9	n.s.
IL	81.8 ± 62.1	41.0 ± 18.3	0.4	n.s.	51.1 ± 11.5	45.7 ± 18.3	0.1	n.s.
EL	484.0 ± 112.7	122.6 ± 41.2	20.9	0.02	386.6 ± 73.4	132.7 ± 60.1	5.8	(0.06)

Animals were tested (15 min) twice weekly. Data were subjected to one-way ANOVA
[1] Various sexual behaviors were analysed: mounts (Mts), intromissions (Int), ejaculations (Ejac), and latencies (sec) to first mount (ML), to first intromission (IL) and first ejaculation (EL)
[2] Mean of two tests (weeks 8 and 9) before 8-OH-DPAT treatment
[3] Mean of two tests (weeks 8 and 10) with 0.2 mg/kg 8-OH-DPAT
[4] Mean of three tests (weeks 11-13) after 8-OH-DPAT treatment
[5] Mean of three tests (weeks 10-12) with 0.4 mg/kg 8-OH-DPAT

Behavioral testing

Behavioral testing was carried out in a semicircular arena, measuring 62x40x36 cm, with a wire mesh floor and a transparent front (e.g., Broere *et al.*, 1985). The test room was dimly illuminated with indirect white light (60W) and testing took place during the first quarter of the animals' dark period.

Twelve male rats were tested with an estrous female for male sexual behavior during 15 min. Testing occurred twice weekly up to week 23 and once weekly during the final 7 weeks. The animals were allowed to become accustomed to the test cage for 15 min before testing. The following behavioral data were collected: number of mounts with pelvic thrusts, intromissions, ejaculations, latency (sec) from the start of test to first mount, to first intromission, to first ejaculation, and number of mounts and intromissions before first ejaculation.

Statistical analysis

Data were subjected to a one way analysis of variance (ANOVA) for repeated measures (Perlman, 1986). When there was a significant overall effect ($p<0.05$), the least significant difference (LSD) test was used to make pairwise comparisons among means (Kirk, 1968).

TABLE 5.3. Effects of two doses of 8-OH-DPAT (0.2 mg/kg in week 23, 0.4 mg/kg in week 22) on sexual behavior (means ± SE) of 12 male rats castrated 20-23 weeks earlier. The animals had subnormal serum levels of testosterone (mean 4.3 nmol/l).

Behavior[1]	saline[2]	8-OH-DPAT 0.2 mg/kg	8-OH-DPAT 0.4 mg/kg	F(4,44)	p	LSD(5%)	LSD(1%)
Mts	8.3 ± 1.1	17.8 ± 3.2	3.2 ± 0.6	8.4	<0.001	3.2	4.7
Int	10.0 ± 0.8	8.3 ± 1.1	8.1 ± 1.0	2.1	n.s.	-	-
Ejac	0.4 ± 0.1	1.2 ± 0.2	2.1 ± 0.1	47.7	<0.001	0.2	0.3
ML	40.8 ± 24.2	8.4 ± 2.6	7.3 ± 1.1	1.5	n.s.	-	-
IL	90.9 ± 31.7	15.1 ± 3.2	9.5 ± 1.0	3.2	<0.05	79.3	-
EL	755.8 ± 43.3	403.8 ± 70.4	100.4 ± 11.5	48.0	<0.001	77.6	113.3
Before 1st ejaculation				F(4,12)			
Mts	6.6 ± 1.6	12.1 ± 4.9	1.8 ± 0.5	1.7	n.s.	-	-
Int	11.1 ± 1.4	7.2 ± 1.3	3.9 ± 0.7	7.4	<0.005	2.4	3.5
Ejaculators only							
ML	17.1 ± 5.2	7.6 ± 3.4	7.6 ± 1.5	1.7	n.s.	-	-
IL	37.7 ± 14.5	13.2 ± 4.2	11.0 ± 0.9	1.8	n.s.	-	-
EL	594.2 ± 52.8	336.4 ± 66.1	92.4 ± 13.2	6.8	<0.005	174.6	255.0

Animals were tested (15 min) twice weekly. Data were subjected to one-way ANOVA
[1] Various sexual behaviors were analysed: mounts (Mts), intromissions (Int), ejaculations (Ejac), and latencies (sec) to first mount (ML), to first intromission (IL) and to first ejaculation (EL)
[2] Mean of two tests before 8-OH-DPAT treatment (weeks 21-22) and one test after 8-OH-DPAT treatment (week 23)

RESULTS

Sexually experienced male rats displayed a relatively high ejaculatory frequency for several weeks after castration. A low ejaculatory frequency was found during week 8 after castration. The effects of 8-OH-DPAT, in doses of 0.2 and 0.4 mg/kg body weight, were studied during weeks 8-12 (figure 5.1).

For weeks 1-6 after castration, statistical analysis (one-way ANOVA) revealed a significant decrease in the frequency of mounts, intromissions and ejaculations, and a significant increase in the latency to first mount, to first intromission and to first ejaculation compared to results obtained before the animals were castrated (block 1; table 5.1).

8-OH-DPAT moderately stimulated these parameters of sexual behavior (compared to saline tests) 2-3 months after castration (figure 5.1 and table 5.2).

The lower dose of 8-OH-DPAT significantly reduced the number of intromissions before first ejaculation and the first ejaculation latency. The number of mounts and intromissions showed a non-significant decrease ($p=0.08$). The higher dose of 8-OH-DPAT induced a significant decrease in

TABLE 5.4. Effects of two doses of 8-OH-DPAT on sexual behavior (means ± SE) of 12 male rats castrated 8-12 weeks before testing.

Behavior[1]	saline[2]	8-OH-DPAT 0.4 mg/kg[3]	F(1,11)	p
Mts	4.0 ± 0.7	3.7 ± 1.2	0.1	n.s.
Int	0.2 ± 0.1	2.2 ± 0.8	7.2	<0.03
Ejac	0.0	0.4 ± 0.2	5.2	<0.05
ML	544.3 ± 40.6	383.7 ± 107.1	2.5	n.s.
IL	854.6 ± 20.5	437.8 ± 115.3	12.8	<0.01
EL	900.0	759.8 ± 68.2	4.2	(0.07)
Before 1st ejaculation				
Mts	-	4.8 ± 1.2	-	
Int	-	3.8 ± 1.0	-	
Ejaculators only				
ML	-	12.8 ± 6.3	-	
IL	-	48.1 ± 25.2	-	
EL	-	468.7 ± 114.6	-	

Animals were tested (15 min) once weekly. Data were subjected to one-way ANOVA
[1] Various sexual behaviors were analysed: mounts (Mts), intromissions (Int), ejaculations (Ejac), and latencies (sec) to first mount (ML), to first intromission (IL) and to first ejaculation (EL)
[2] Mean of two tests before 8-OH-DPAT treatment (weeks 48 and 50) and one test after 8-OH-DPAT treatment (week 52)
[3] Mean of two tests after 8-OH-DPAT treatment (weeks 49 and 51)

intromission and ejaculation latencies, and also in the number of intromissions before first ejaculation. There was a non-significant (p=0.08) stimulatory effect on the number of ejaculations and a non-significant (p=0.06) decrease in ejaculation latency.

In weeks 17 and 18 after castration, the higher dose 8-OH-DPAT no longer exerted a stimulatory effect on male sexual behaviors (figure 5.1). A small testosterone-filled Silastic capsule was then implanted, which resulted in serum testosterone levels of 4.6 ± 0.04 nmol/l (mean ± SE). The levels are lower than those of normal rats (Damassa *et al.*, 1977). Under these conditions, clear-cut stimulatory effects of 8-OH-DPAT compared to saline were found (figure 5.1, table 5.3). There was a significant increase in the number of mounts and ejaculations, a decrease in the number of intromissions before first ejaculation, and a decrease in the latencies to first intromission and ejaculation. Serum testosterone levels 4 weeks after capsule implantation were 4.1 ± 0.4 nmol/l.

After 5 months without behavioral tests, testosterone levels ranged from 0.9 ± 0.1 nmol/l (week 46 postcastration) to 0.6 ± 0.1 nmol/l (week 52). 8-OH-DPAT (0.4 mg/kg), compared to saline, significantly increased the number of intromissions and ejaculations and decreased the intromission latency and ejaculation latency (figure 5.1, table 5.4).

DISCUSSION

The main findings of this study can be summarized as follows. Without testosterone substitution and between 8-12 weeks after castration, 8-OH-DPAT had marginal stimulatory effects on the sexual behavior of male rats. Major stimulatory effects of 8-OH-DPAT on sexual behavior (i.e. increase in the frequency of the behaviors and in the number of animals copulating) in castrated male rats tested 17-52 weeks after castration seemed to require the presence of testosterone in the blood, with low levels such as 0.6 nmol/l being sufficient.

Thus, minimal levels of testosterone seem to be required for 8-OH-DPAT to affect the sexual behavior of male rats one year after castration. At 8-12 weeks after castration 8-OH-DPAT significantly reduced the number of intromissions before first ejaculation only. Although not measured in the present study, testosterone was probably present in the serum, albeit in very low concentrations (≤0.4 nmol/l), as evidenced by recent data published by Meijers and coworkers (1992). These authors found endogenous testosterone levels to be virtually undetectable 16 weeks after castration (0.14 nmol/l). In the present study when castrated rats ceased to copulate, i.e. during weeks 17 and 18, we found that 8-OH-DPAT no longer stimulated male sexual behavior, presumably because endogenous testosterone levels were too low.

From the present study it also appeared that handling and injecting the animals before testing increased sexual activity, as shown by the data for saline-injected rats in weeks 7 and 8 after castration. We have no explanation for this interesting finding.

We have previously found that 8-OH-DPAT only stimulates mounting behavior in ovariectomized female rats when they are simultaneously treated with testosterone (chapter 4, exp. 4.4). Ahlenius *et al.* (1981) suggested that 8-OH-DPAT could completely restore sexual behavior in castrated male rats. However, these researchers used rats that had been castrated about 1 month earlier and which had no sexual experience before castration; the animals were tested only 5 times. They found that 8-OH-DPAT-treated male rats differed from the saline-treated control rats, which did not display sexual behavior. The latter finding is different from most studies because many authors report that male rats show copulatory behavior up to 6 weeks after castration (e.g., Beach, 1944, Davidson, 1966). We suggest therefore, that Ahlenius *et al.* (1981) should have waited

longer after castration before testing the possible stimulatory properties of 8-OH-DPAT in male rats. The longer time is also required for endogenous testosterone levels to drop to extremely low or undetectable levels (e.g., Meijers, 1992).

In the light of the results of the present study, the findings of Clark *et al.* (1985) need to be discussed. These investigators reached the conclusion that testosterone was not required for the enhancement of male rat sexual behavior by yohimbine. They tested rats 35, 56 and 91 days after castration, times at which rats can still be sexually active, as can be seen from the present study. It remains to be demonstrated if yohimbine can stimulate sexual behavior in male rats 17 weeks or more after castration. In the present study, 8-OH-DPAT could not stimulate sexual behavior at these times. The similarities between our experiment and that of Clark and coworkers (1985) can be found in the stimulatory effects of both 8-OH-DPAT and the alpha$_2$ adrenoceptor antagonist yohimbine. It has been suggested that the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT also activates alpha$_2$ receptors (Winter, 1988, Schnur *et al.*, 1989).

When the rats were tested regularly (twice weekly), they continued to display all parameters of sexual behavior for many weeks after castration. There was a gradual decline in the frequency of ejaculation. The number of intromissions before first ejaculation decreased abruptly in the first week after castration and remained stable for many weeks thereafter, with unchanged mean inter-intromission intervals. This interesting phenomenon was found and discussed by Davidson (1966). Since then, no new findings have emerged to explain this interesting occurrence.

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SUMMARY

The stimulatory effects of 8-OH-DPAT on male sexual behavior in the absence and presence of testosterone were investigated. Male rats (n=12) were castrated

and tested for sexual behavior (15 min, with an estrous female) up to 1 year after castration. Castration caused an abrupt decrease (50%) in the number of intromissions before first ejaculation, and the number remained stable for about 8 weeks. Between 8-12 weeks after castration, when ejaculation frequency was low, 0.2 or 0.4 mg/kg 8-OH-DPAT had no effect on ejaculation frequency, but significantly decreased the number of intromissions before first ejaculation. In weeks 17-18 after castration, 0.4 mg/kg 8-OH-DPAT no longer affected copulatory behavior. A 5 mm testosterone filled Silastic capsule (implanted at week 19 after castration) resulted in subnormal plasma testosterone levels (mean 4.4 nmol/l) and did not fully restore male copulatory behavior. Administration of 8-OH-DPAT (0.2 and 0.4 mg/kg) was followed by an increase in ejaculation frequency and a decrease in ejaculation latency. Five months later, when plasma testosterone levels were very low (mean 0.6 nmol/l), 8-OH-DPAT (0.4 mg/kg) significantly increased the mean number of intromissions and ejaculations and decreased the number of intromissions before first ejaculation, intromission latency and ejaculation latency (borderline). The present results suggest that testosterone is required for the activating effects of 8-OH-DPAT on sexual behavior in castrated male rats, tested 17-52 weeks after castration.

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Chapter 6

Flesinoxan: a prosexual drug for male rats*

THE NEUROTRANSMITTER SEROTONIN (5-hydroxytryptamine, 5-HT) has been known for its involvement in male rat sexual behavior since the late sixties (e.g., Tagliamonte *et al.*, 1969, Gessa and Tagliamonte, 1974). By now, there is quite an extensive literature on the proclaimed stimulatory effects of serotonergic agents, especially the various 5-HT_{1A} receptor agonists (e.g., 8-hydroxy-2-(di-propylamino) tetralin (8-OH-DPAT), buspirone, ipsapirone, flesinoxan) on male rat sexual behavior (Ahlenius and Larsson, 1988, Mos *et al.*, 1990). The 5-HT_{1A} receptors are somatodendritic and terminal autoreceptors in the central nervous system, with the highest density in the dorsal raphe nuclei (Marsden and Kendall, 1992). Facilitation of these autoreceptors results in an inhibition of neuronal firing of rat raphe neurons, and subsequently in a fall of extracellular 5-HT in the striatum and ventral hippocampus (Marsden and Kendall, 1992, Kreiss and Lucki, 1994).

In male rats 8-OH-DPAT increases ejaculation frequency, but concomitantly decreases intromission frequency (Ahlenius *et al.*, 1981, chapter 4). Quite often such ejaculation behavior resulted in abnormal deposition of the ejaculate, suggesting that no proper penile intromission had occurred (chapter 4, experiment 4.2). Therefore, we earlier suggested that 8-OH-DPAT renders male rats to become "premature" ejaculators (chapter 4), which could plea against naming this drug "prosexual" (i.e. stimulating sexual behavior without altering the specific pattern of male rat sexual behaviors, for instance more animals to be sexually active, higher ejaculation frequencies, shorter latencies to ejaculation, shorter post-ejaculatory intervals, etc.; Kwong *et al.*, 1986, Mos *et al.*, 1990).

Flesinoxan, also a selective 5-HT_{1A} receptor agonist, is reported to react similarly as 8-OH-DPAT in behavioral and pharmacological male rat studies

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(Mos *et al.*, 1990, Ybema *et al.*, 1990, Ahlenius *et al.*, 1991). However, critical reading of these studies indicates differences from 8-OH-DPAT: flesinoxan also increases ejaculation frequency but does not significantly affect other sexual behaviors. The present study was designed to investigate this in more detail.

The presumed prosexual properties of flesinoxan were studied in control rats and in male rats with impaired sexual behavior. Impaired sexual behavior can be found in male rats which were neonatally deprived of endogenous estrogen (Vreeburg *et al.*, 1977, Davis *et al.*, 1979). Such deprivation can be achieved by treating newborn male rats with the aromatase inhibitor ATD (1,4,6-androstatriene-3,17-dion), which blocks the aromatization of testicular testosterone to estradiol in specific areas of the central nervous system (Bakker *et al.*, 1996). In adulthood, such ATD-males show normal frequencies of mounts and intromissions, but no or very low frequencies of ejaculation behavior when tested early in the dark phase with a female rat in heat (Bakker *et al.*, 1993).

GENERAL METHOD

Animals and treatments

Female albino Wistar rats (HSD, Zeist, The Netherlands) were time mated. Within 3 h after birth male pups received subcutaneously in the neck under hypothermic anesthesia a silastic implant (inner diameter 1.5 mm, outer diameter 2.1 mm, effective length 5 mm) randomly filled with either ATD or left empty (placebo group). At 21 days of age, the implants were removed, the animals weaned and housed 2-3 of same sex and treatment to a cage with food and water available *ad lib.* In adulthood, these males were behaviorally tested. Earlier studies revealed that adult ATD and control males have similar serum levels of endogenous testosterone, FSH, LH and inhibin (Bakker *et al.*, 1995). The day-night cycle was artificially maintained (dark: 5:30 p.m. - 7:30 a.m.). Temperature in the air-conditioned room ranged from 20 to 22°C.

Adult stimulus female rats were ovariectomized via bilateral incisions under ether anesthesia. They were brought into behavioral estrus by injecting 20 μg estradiol benzoate in 0.1 ml olive oil 24 h prior to testing, followed by 2.5 mg progesterone in 0.1 ml oil 3-4 h before testing.

A fresh solution of flesinoxan (kindly provided by Dr J. Mos, Solvay-Duphar, Weesp, The Netherlands) or 8-OH-DPAT ((±) 8-hydroxy-2-(di-propylamino)

tetralin·HBr; Research Biochemicals Inc., Natick, MA) was dissolved in saline approximately 1 h before testing to a volume of 1 ml/kg body weight. Flesinoxan was administered intraperitoneally (i.p.) and 8-OH-DPAT was given subcutaneously (SC).

Behavioral testing

Pair-tests with an estrous female were carried out in a semicircular arena, measuring 62 x 40 x 36 cm, with a wire mesh floor and a transparent front. Testing started about 1 h after the onset of the dark cycle and the males were allowed to adapt to the test cage for at least 15 min. The test room was dimly illuminated with indirect white light. At least 3 pair-tests preceded the onset of experimental testing.

The following sexual behaviors were recorded: mount latency: time from start of test till first mount with pelvic thrusts; intromission latency: time from start of test till first intromission; ejaculation latency: time from first mount or intromission till first ejaculation; mounts: number of mounts with pelvic thrusts; number of intromissions; number of ejaculations; post-ejaculatory interval: time from ejaculation to first following sexual behavior.

Statistical analysis

The data were subjected to two- or three-way analyses of variance (ANOVA) for repeated measures (Perlman, 1986). If there was an overall statistically significant effect, the Least Significant Difference (LSD) Test was used to make pairwise comparisons amongst means (Kirk, 1968). The 0.05 level of probability (2-tailed) was adopted as the level of statistical significance.

EXPERIMENT 6.1 - FLESINOXAN AND SEXUAL BEHAVIOR IN ATD-MALE RATS AND CONTROLS

It was earlier reported that 0.2 and 0.4 mg/kg 8-OH-DPAT increased ejaculation frequencies in ATD-males (Brand *et al.*, 1991). Ahlenius *et al.* (1991) reported facilitation of ejaculatory behavior by flesinoxan similar to the effect of 8-OH-DPAT in normal male rats. Present experiment 6.1 was carried out to investigate the behavioral effects of flesinoxan (two doses) in ATD-males and controls.

Method

Twenty-three heterosexually experienced male rats were used, 12 controls and 11 ATD-males, aged 47 weeks, with a mean body weight of 490 g (range: 375-575 g). In six weekly pair-tests the animals received consecutively: nothing, saline, 0.3 mg/kg flesinoxan, saline, 1.0 mg/kg flesinoxan and saline. The drug or vehicle was administered intraperitoneally, in a volume of 1 ml/kg. Behavioral testing lasted 15 min.

Results

As can be learned from table 6.1, all sexual behaviors except mount latency showed a statistical significant difference over the tests. Most striking effects were found with flesinoxan treatment in ejaculation behavior: almost all animals displayed the behavior. With flesinoxan the mean frequencies of ejaculations per test increased and the mean latencies to first ejaculation decreased in both groups. Although not statistically different (LSD(5%)= 1.1), highest mean ejaculation frequencies were seen with flesinoxan treatment (0.3 and 1.0 mg/kg) both in ATD males and controls. The number of animals ejaculating increased during flesinoxan treatment: with low dose 9 of 11 (82%) ATD-males, and 11 of 12 (92%) controls, and with the high dose all animals ejaculated. In the blank and saline tests clearly less ATD-males displayed ejaculatory behavior. Post-hoc analysis with McNemar's test revealed that with 1.0 mg/kg flesinoxan significantly more ATD-males ejaculated (11 of 11) than in the blank test (3 of 11; $P<0.01$) and than in the first saline test (5 of 11; $P<0.05$).

The shortest latencies to first ejaculation were found in both groups during flesinoxan treatment. This effect was most outspoken in the ATD-males. Post-hoc analysis revealed that during flesinoxan treatment (0.3 and 1.0 mg/kg) the latencies to first ejaculation no longer differed between controls and ATD-males (LSD(5%)= 137). Combining data of ATD-males and controls, shortest latencies (mean = 278 s) were found with 1.0 mg/kg versus 360 s with 0.3 mg/kg treatment, but this difference was not statistically significant (LSD(5%)=314).

To investigate a possible period effect of testing, a subsequent 2-way analysis of variance was performed on the 3 saline tests only. No statistical significant differences were found in latencies. There was an effect of groups ($p<0.04$) but not of tests in the number of ejaculations. In the ATD group, the number of animals ejaculating did not differ between the first and third saline test (5/11 vs.

TABLE 6.1. Experiment 6.1. Effects of two doses of flesinoxan (mean ± SE (n)) on sexual behavior of male rats: 12 control Wistar rats (contr) and 11 rats neonatally treated with ATD

Behavior[1]	Group	Consecutive treatments: blank	saline	flesinoxan 0.3 mg/kg	saline	flesinoxan 1.0 mg/kg	saline	Statistics: two-way ANOVA p
Mts	Contr	25.9 ± 4.2 (12)	24.2 ± 5.2 (12)	22.9 ± 3.6 (12)	13.4 ± 2.2 (12)	14.1 ± 2.2 (12)	14.9 ± 2.6 (12)	test <0.001
	ATD	19.9 ± 2.0 (11)	17.8 ± 2.1 (11)	25.5 ± 3.1 (11)	12.2 ± 3.7 (11)	16.8 ± 1.9 (11)	10.1 ± 1.7 (11)	
Int	Contr	10.8 ± 1.5 (12)	15.8 ± 1.2 (12)	13.2 ± 1.5 (12)	11.2 ± 1.6 (11)	14.2 ± 1.5 (12)	14.5 ± 1.8 (12)	test: 0.007
	ATD	11.3 ± 2.0 (11)	14.5 ± 1.8 (11)	12.7 ± 1.0 (11)	12.3 ± 1.5 (11)	12.6 ± 1.3 (11)	14.4 ± 1.1 (11)	
Ejacs	Contr	1.0 ± 0.2 (9)	1.8 ± 0.2 (11)	2.2 ± 0.3 (11)	1.8 ± 0.3 (10)	2.3 ± 0.2 (12)	2.2 ± 0.3 (10)	test: <0.001
	ATD	0.5 ± 0.2 (3)	0.9 ± 0.3 (5)	1.7 ± 0.4 (9)	1.1 ± 0.3 (6)	1.7 ± 0.2 (11)	1.3 ± 0.4 (8)	group: 0.03
ML	Contr	9.5 ± 2.1	6.4 ± 1.1	6.2 ± 0.8	21.8 ± 13.2	5.2 ± 1.0	4.8 ± 0.8	test:: (0.08)
	ATD	5.6 ± 0.7	6.0 ± 0.7	7.0 ± 1.6	13.5 ± 6.9	5.3 ± 1.5	8.8 ± 3.5	
IL[2]	Contr	47.6 ± 17.7	13.8 ± 3.6	20.1 ± 5.9	119.8 ± 73.4	13.0 ± 4.7	10.5 ± 2.9	test: 0.02
	ATD	77.1 ± 32.2	23.8 ± 14.7	19.3 ± 5.2	93.3 ± 75.4	7.6 ± 1.2	15.2 ± 5.5	
EL[2]	Contr	600 ± 74	326 ± 62	339 ± 87	365 ± 81	222 ± 32	339 ± 79	test: <0.001
	ATD	748 ± 79	641 ± 106	381 ± 95	520 ± 113	333 ± 69	474 ± 92	gr: (0.07)

[1] Various sexual behaviors were analysed: number of mounts (mts), intromissions (int), ejaculations (ejacs) and latencies (sec) to first mount (ML), to first intromission (IL) and to first ejaculation (EL). Animals were pair-tested in a semicircular arena, 30 min after intraperitoneal drug administration. [2] non-responders: 900 sec

8/11, Fisher exact probability test, n.s.). The number of mounts was significantly higher in the first saline test in both control and ATD-males than the two subsequent saline tests ($p=0.002$). With respect to the number of intromissions during the 3 saline tests the only significant difference appeared between first and second saline test in both groups of males ($p<0.02$); the first and second saline test did not differ from the third test.

Surprisingly, during flesinoxan treatment, none of the males ejaculated "prematurely," i.e. during the first or second intromission. To investigate this phenomenon in more detail, a second experiment was performed.

EXPERIMENT 6.2 - A COMPARISON BETWEEN THE EFFECTS OF 8-OH-DPAT AND FLESINOXAN ON SEXUAL BEHAVIOR IN ATD-MALES AND CONTROL RATS

From experiment 6.1 it became clear that flesinoxan had prosexual (i.e. sexually stimulating) effects in adult male rats. The stimulatory effects seemed different from what has been described for 8-OH-DPAT: with flesinoxan the rats showed higher frequencies and shorter latencies of sexual behaviors, and 8-OH-DPAT

rendered them into "premature" ejaculators (chapter 4). Experiment 6.2 was carried out to compare the behavioral effects of 0.4 mg/kg 8-OH-DPAT and flesinoxan (in 3 different doses) in control ATD-males. The dose of 0.4 mg/kg was chosen on the basis of various earlier experiments (Brand *et al.*, 1991, Mos *et al.*, 1990, chapters 4 and 6). Testing lasted normally till the second ejaculation. This enabled us to study the presumed stimulatory effects of both 5-HT_{1A} receptor agonists in two consecutive ejaculation series.

Method

Nine sexually experienced ATD-male rats and ten controls (aged 5 months, mean body weight 355 g, range: 295-395 g) were pair-tested once weekly with an estrous female and received various consecutive drug treatments 30 min prior to testing: blank, 0.4 mg/kg 8-OH-DPAT SC., 1 ml/kg saline SC., 0.3 mg/kg flesinoxan i.p., 1.0 mg/kg i.p., 3.0 mg/kg flesinoxan i.p., 1 ml/kg saline i.p.. Animals were tested till second ejaculation or for 25 min. The first ejaculation series is defined as the time from start of testing till first ejaculation. The second ejaculation series is defined as the time from first sexual behavior after first ejaculation till second ejaculation. Between first ejaculation and the onset of the second ejaculation series is the (first) refractory period.

Results

Various sexual behaviors are presented in table 6.2. All control male rats ejaculated twice in each test. Only during 5-HT_{1A} receptor agonist treatment all ATD-males ejaculated twice. From inspecting the data it is clear that 5-HT_{1A} receptor agonist treatment affected sexual behaviors of ATD and control males, both during first and second ejaculation series. In general, mount and intromission frequencies were lower, and mount, intromission and ejaculation latencies were shorter during 5-HT_{1A} receptor agonist treatment than during blank or saline treatment. Because the objective of this experiment was to compare the effects of two different 5-HT_{1A} receptor agonists, these data were subjected to 3-way analysis of variance, with factors groups, test and ejaculation series (see figure 6.1 and table 6.3).

When comparing 8-OH-DPAT with flesinoxan treatment, some interesting differences were found. During the first ejaculation series (hatched bars) the differences between 8-OH-DPAT and flesinoxan are most obvious, both in

TABLE 6.2. Experiment 6.2. Various sexual behaviors (mean ± SE (n)) of male rats: 10 control Wistar rats (contr) and 9 rats neonatally treated with ATD

Behavior		blank (n)[2]	8-OH-DPAT 0.4 mg/kg	saline	flesinoxan 0.3 mg/kg	flesinoxan 1.0 mg/kg	flesinoxan 3.0 mg/kg	saline
FIRST EJACULATION SERIES[3]								
Mts	Contr	13.9 ± 1.8	0.5 ± 0.2	11.8 ± 1.8	7.0 ± 1.2	7.0 ± 1.2	3.5 ± 0.7	10.1 ± 2.9
	ATD (ejac only)[4]	25.0 ± 0.7 (2)	1.1 ± 0.6	20.5 ± 3.0 (6)	8.9 ± 1.3	8.3 ± 2.4	2.3 ± 0.8	13.1 ± 3.2 (8)
	ATD (all)	23.2 ± 3.9		24.0 ± 2.0				16.6 ± 2.8
Int	Contr	10.9 ± 1.0	0.3 ± 0.2	13.5 ± 1.1	10.9 ± 1.3	9.6 ± 1.2	6.5 ± 1.1	12.3 ± 1.6
	ATD (ejac only)	14.0 ± 1.4 (2)	0.8 ± 0.3	19.0 ± 1.9 (6)	13.8 ± 1.4	7.7 ± 1.4	5.4 ± 0.8	14.1 ± 1.9 (8)
	ATD (all)	13.7 ± 2.5		21.3 ± 1.7				18.4 ± 1.7
ML	Contr	24.2 ± 9.5	17.8 ± 2.7	18.9 ± 8.0	7.4 ± 1.9	8.4 ± 3.5	6.1 ± 1.5	9.7 ± 3.8
	ATD (all)	25.1 ± 15.4	11.6 ± 1.6	15.0 ± 6.0	7.3 ± 2.8	13.0 ± 4.3	23.4 ± 7.4	15.4 ± 5.5
IL	Contr	39.5 ± 9.4	21.1 ± 2.9	27.5 ± 9.2	15.6 ± 4.4	16.6 ± 3.9	15.0 ± 4.2	19.3 ± 3.5
	ATD (all)	90.7 ± 46.2	34.4 ± 18.1	22.4 ± 5.8	10.9 ± 3.0	19.0 ± 6.6	31.8 ± 11.2	39.9 ± 19.0
EL	Contr	412 ± 51	13 ± 7	413 ± 37	201 ± 22	161 ± 24	129 ± 23	382 ± 63
	ATD (ejac only)	536 ± 99 (2)	47 ± 20	825 ± 128 (6)	337 ± 54	210 ± 41	103 ± 10	664 ± 125 (8)
	ATD (all)[5]	> 1264		> 1043				> 756
PEI[5]	Contr	416 ± 10	462 ± 18	307 ± 15	263 ± 9	230 ± 11	314 ± 23	286 ± 19
	ATD (ejac only)	390 ± 51 (2)	222 ± 20	265 ± 20 (6)	214 ± 7	152 ± 22	160 ± 20	276 ± 23 (8)
SECOND EJACULATION SERIES[7]								
Mts	Contr	11.2 ± 1.6	2.6 ± 0.4	13.0 ± 3.2	4.7 ± 0.9	3.8 ± 0.8	2.9 ± 0.4	6.8 ± 1.5
	ATD (ejac only)	6.0 ± 2.1 (2)	3.4 ± 0.5	7.3 ± 2.2 (3)	6.3 ± 1.2	7.7 ± 2.4	4.0 ± 0.5	5.0 ± 1.3 (5)
Int	Contr	6.4 ± 0.9	2.8 ± 0.3	6.4 ± 0.9	5.2 ± 0.7	3.7 ± 0.4	3.4 ± 0.3	5.6 ± 0.8
	ATD (ejac only)	6.0 ± 0.7 (2)	2.8 ± 0.3	4.0 ± 0.9 (3)	6.1 ± 0.5	5.7 ± 0.8	4.3 ± 0.8	5.4 ± 0.4 (5)
EL	Contr	269 ± 26	134 ± 20	276 ± 60	113 ± 18	84 ± 8	117 ± 22	153 ± 23
	ATD (ejac only)	165 ± 0 (2)	178 ± 36	203 ± 11 (3)	215 ± 23	243 ± 55	151 ± 22	190 ± 24 (5)

[1] Various sexual behavior were analysed: number of mounts (mts), intromissions (int), post-ejaculatory interval (PEI) and latencies to first mount (ML), to first intromissions (IL) and to first ejaculation (EL). were pair-tested in a semicircular arena, 30 min after drug administration. Animals were tested with an estrous female during 25 min, or till second ejaculation occurred. [2] Number of animals displaying the behavior. Indicated only if non-responders were present. [3] Time from start of test till first ejaculation. [4] ejac only: ejaculators only. [5] Non-ejaculators: 1500 sec. [6] Post-ejaculatory interval (PEI): time between first and onset of second ejaculation series. [7] Time from first sexual behavior after first ejaculation to second ejaculation.

control and ATD-males. Compared to flesinoxan, lower mount and intromission frequencies and a shorter ejaculation latency to first ejaculation were found with 8-OH-DPAT (figure 6.1).

During the first ejaculation series there appeared to be a dose dependent effect of flesinoxan: with higher doses of flesinoxan there was a decrease in mean number of mounts (LSD(5%)=1.9), of intromissions (LSD(5%)=1.1) and in mean ejaculation latency (LSD(5%)=41) in all cases. Post-hoc analyses revealed a significant effect for all behaviors with 0.3 vs 3.0 mg/kg flesinoxan, and for all three doses of flesinoxan with intromissions prior to first ejaculation.

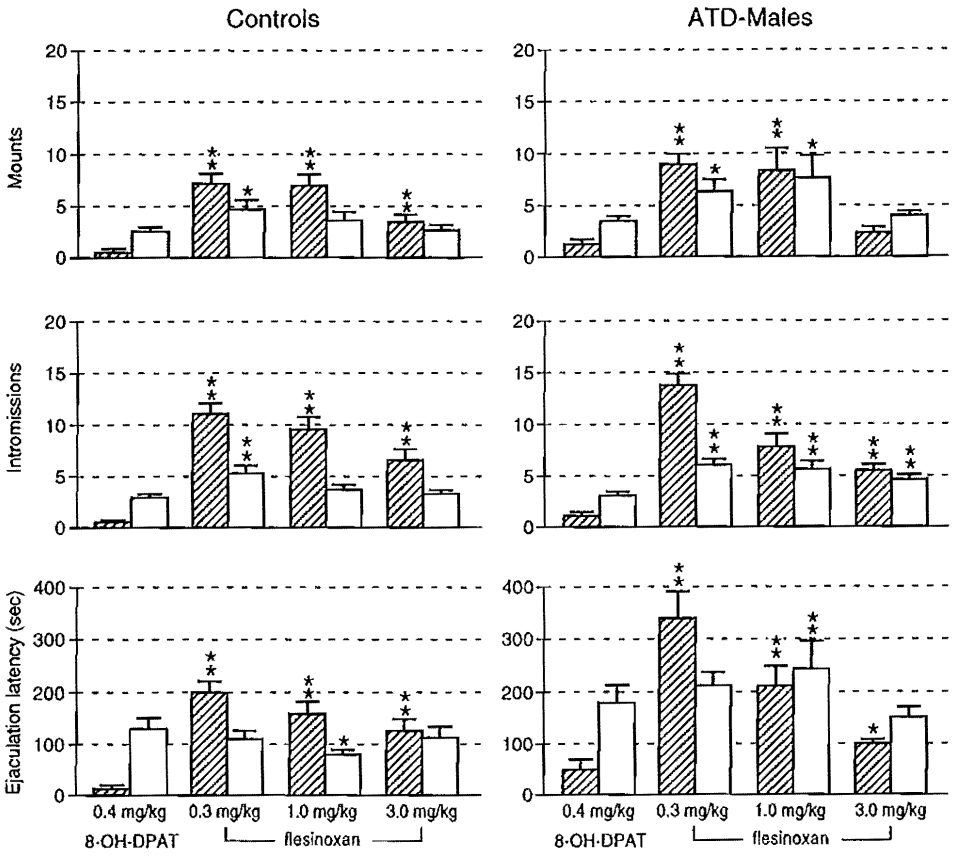

FIGURE 6.1. Various sexual behaviors (mean + SE) of 2 groups of male rats: controls (n=10) and neonatally ATD treated males (n=9) during first (hatched bars) and second (open bars) ejaculation series. Asterisks indicate a statistical significant difference (*P<0.05, **P<0.01) between flesinoxan and 8-OH-DPAT treatment, per group and per ejaculation series. Animals were pair-tested with an estrous female until second ejaculation. Drugs were administered 30 min before testing.

Ejaculation with the first or second intromission (i.e. "premature" ejaculations) occurred only with 8-OH-DPAT treatment and only during the first ejaculation series in 8 of 10 control males, and in 6 of 9 ATD-males. Premature ejaculations were not observed with 0.3, 1.0 or 3.0 mg/kg flesinoxan treatment.

With regard to the post-ejaculatory interval after first ejaculation it was found that there was a difference of groups ($p<0.001$), of tests ($p<0.001$) and a significant group x test interaction ($p<0.001$). With 0.4 mg/kg 8-OH-DPAT controls had a post-ejaculatory interval that was more than twice as long as ATD males ($p<0.001$), whereas there was no significant difference between the groups with 0.3 mg/kg flesinoxan (LSD(5%)=45: n.s.).

TABLE 6.3. Statistical results of data collected in experiment 6.2.

3-way ANOVA		Prior to ejaculation		Ejaculation
Factor		Mounts: p	Intromissions: p	Latency: p
Test (t)		< 0.001	< 0.001	< 0.001
Group (g)		n.s.	n.s.	0.019
1st vs. 2nd ejaculation series		n.s.	<0.001	n.s.
Interactions:	t x g	n.s.	n.s.	0.026
	t x e	< 0.001	<0.001	<0.001
	g x e	n.s.	n.s.	0.069
	t x g x e	n.s.	0.054	n.s.

3-way analyses of variance were performed on tests with flesinoxan and 8-OH-DPAT only. Two-tailed p-values of ≤ 0.10 are indicated. P values of ≤ 0.05 are considered to be statistically significant.

During the second ejaculation series (open bars) the mean differences between 8-OH-DPAT treatment and flesinoxan were smaller (figure 6.1). In mean number of mounts prior to second ejaculation, 0.3 mg/kg flesinoxan differed from all other treatment tests in control rats; in ATD-males, 0.3 and 1.0 mg/kg flesinoxan differed from 0.4 mg/kg 8-OH-DPAT and 3.0 mg/kg flesinoxan (LSD(5%)=1.9). When comparing the mean number of intromissions prior to second ejaculation, 0.4 mg/kg 8-OH-DPAT differed from 0.3 mg/kg flesinoxan in control rats, and from all doses of flesinoxan treatment in ATD-males (LSD(5%)=1.1). Latency to second ejaculation was significantly longer with 1.0 mg/kg flesinoxan than with 0.4 mg/kg 8-OH-DPAT in both groups (LSD(5%)=41). In ATD-males, 0.3 mg/kg flesinoxan also differed from 3.0 mg/kg flesinoxan.

DISCUSSION

The main findings of the two experiments can be summarized as follows. Flesinoxan treatment stimulated ejaculation frequency and decreased ejaculation latency in normal male rats and in males with impaired sexual behavior (neonatal ATD treatment). This was in line with results of earlier experiments with another 5-HT_{1A} receptor agonist, 8-OH-DPAT (Ahlenius *et al.*, 1991, Brand *et al.*, 1991, chapter 4). With flesinoxan treatment, sexual behavior of ATD-males did not differ from control rats. Surprisingly, with flesinoxan no "premature" ejaculations (i.e. ejaculation with the first or second intromission) were observed in neither group in both experiments. The most striking differences between 8-OH-DPAT and flesinoxan appeared during the first ejaculation series: nine of ten

controls and six of nine ATD males ejaculated "prematurely" with 8-OH-DPAT but none of the animals did so with flesinoxan. When looking at the second ejaculation series, the differences between 8-OH-DPAT and flesinoxan were remarkably smaller. The most striking differences between 8-OH-DPAT and flesinoxan on the stimulatory properties on male rat sexual behavior were seen during the first ejaculation series. For example, in control animals, mounts, intromissions and ejaculation latency were significantly higher with the highest dose of flesinoxan than with 8-OH-DPAT during the first ejaculation series, but were not significantly different during the second ejaculation series.

When testing moderately experienced rats, Mos *et al.* (1990) found similar results for the effect of 8-OH-DPAT and flesinoxan on sexual behavior in male rats. Administration of various serotonergic drugs, including 8-OH-DPAT and flesinoxan, resulted in an increase of ejaculation frequencies, and a decrease of ejaculation latency and mount and intromission frequencies prior to ejaculation. Compared to flesinoxan, the effect of 8-OH-DPAT appeared to be "stronger." Unfortunately, Mos *et al.* (1990) gave no details on how many rats ejaculated at the first or second intromission with the different drugs, and only the first ejaculation series was described.

Ahlenius *et al.* (1991) concluded that flesinoxan and 8-OH-DPAT facilitated sexual behavior in a similar way, with flesinoxan being about an order of magnitude less potent than 8-OH-DPAT. This differs from the present study on some major points (e.g., intromissions prior to first ejaculation, post-ejaculatory interval). There is no easy explanation for these differences: animal strains, housing, testing conditions and drug doses were all comparable. The only difference between the two studies is the administration route of flesinoxan: intraperitoneally in the present study versus subcutaneously by Ahlenius *et al.* (1991).

The difference in facilitation of male rat sexual behavior between the two selective 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT can possibly be explained by an administration route effect or a different pharmacological affinity pattern. The latter possibility would imply a role for dopamine, since flesinoxan has a limited co-affinity for the D_2 receptor (Olivier *et al.*, 1995). Administration of various dopamine receptor agonists facilitate several aspects of copulatory behavior and *ex copula* genital responses (e.g., Napoli-Farris *et al.*, 1984, Bittern and Hull, 1987). When testing male rat sexual behavior with a selective dopamine D_2 receptor agonist, like SDN 919 (pramipexol) there was a significant decrease of mounts and intromissions prior to ejaculation, and of

ejaculation latency in normal male rats. (Ferrari and Giuliani, 1994). However, none of the studies report ejaculations at the first or second intromission with dopaminergic agents. The stimulatory properties of flesinoxan on both the 5-HT_{1A} autoreceptor and the D_2 receptor could work synergistically to decrease ejaculation latency, and the (limited) affinity for the dopamine D_2 receptor of flesinoxan could prevent male rats from ejaculating prematurely. Recently, 8-OH-DPAT is described to have a limited co-affinity for the 5-ht_7 receptor, which is mainly located in the rat hypothalamus (Sleight *et al.*, 1995). To our knowledge, no selective 5-ht_7 receptor agonists have been described.

Administration of 8-OH-DPAT renders male rats to become premature ejaculators: very short latency to ejaculation, and often ejaculation during the first or second intromission (chapter 4, experiment 4.2, present study). From the present study, flesinoxan could be considered a prosexual drug for male rats: normal sexual behavioral pattern, shorter latencies, higher ejaculation frequency and a shorter post-ejaculatory interval.

Recently, several studies have been published on the treatment of premature ejaculation in humans with (selective) serotonin reuptake inhibitors like clomipramine (Althof *et al.*, 1995, chapter 9), paroxetine (Waldinger *et al.*, 1994, Ludovico *et al.*, 1996), and fluoxetine (Graziottin *et al.*, 1996, Lee *et al.*, 1996). Behavioral experiments with selective 5-HT reuptake inhibitors on rats have shown inhibition of male rat sexual behavior (Yells *et al.*, 1994), a long-lasting decrease of the ejaculatory response after a single dose of fluoxetine (Rényi, 1986) and increase of intermount-bout intervals, time-outs, grooming time, ejaculation latency, number of mounts per mount bout, and number of mount bouts prior to ejaculation (Yells *et al.*, 1995). Hence, the administration of serotonin reuptake inhibitors results in a higher concentration of 5-HT in the synapse with a decrease of various ejaculatory behaviors, and administration of 5-HT_{1A} receptor agonists results in a lower concentration of 5-HT in the synapse (Kreiss and Lucki, 1994) with a subsequent increase in ejaculatory behaviors. Apparently, the 5-HT levels in the synapse determine the ejaculatory latency. It remains to be demonstrated whether or not the effects on sexual behavior of 8-OH-DPAT and flesinoxan in male rats could be antagonized by simultaneous administration of a selective serotonin reuptake inhibitor.

Flesinoxan is currently being tested in humans on neuroendocrinological effects and body temperature (e.g. De Koning and De Vries, 1995), on depression (e.g. Grof *et al.*, 1993) and suicidal behavior (Pitchot *et al.*, 1995). From the present

study it is clear that these human studies should also investigate possible effects on the sexual behavior of the male subjects.

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SUMMARY

Two tests were carried out to compare the stimulatory (i.e. prosexual) effects of the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT on sexual behavior in male rats. Two groups of rats were used: normal males and males with impaired masculine sexual behavior due to neonatal treatment with the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD). In experiment 6.1, flesinoxan (0.3 and 1.0 mg/kg) stimulated ejaculation frequency and number of animals displaying this behavior, both in controls and ATD-males. With 0.3 mg/kg flesinoxan ATD-males did not differ from controls in ejaculation frequencies. There was a concomitant decrease in ejaculation latency. No "premature" ejaculations (i.e. at first or second intromission) were observed. In experiment 6.2, the effects of 0.4 mg/kg 8-OH-DPAT, 0.3, 1.0 and 3.0 mg/kg flesinoxan and saline were tested in two ejaculation series. "Premature" ejaculations only occurred during first ejaculation series with 8-OH-DPAT in 8 of 10 controls and in 6 of 9 ATD-males; it did not occur during flesinoxan treatment nor in the 2nd ejaculation series with 8-OH-DPAT treatment. Thus, flesinoxan stimulates sexual behavior in control rats and in rats with impaired sexual behavior. Unlike 8-OH-DPAT flesinoxan doesn't render them into "premature" ejaculators. Therefore, flesinoxan could be considered a prosexual drug for male rats.

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PART 2

HUMAN STUDIES

Chapter 7

Premature ejaculation and serotonin reuptake inhibitors

PREMATURE EJACULATION (PE, rapid ejaculation, *ejaculatio praecox*) is a common male dysfunction in which ejaculation/orgasm occurs before the individual desires due to the absence of voluntary control during sexual activity. Although different authors use different definitions for premature ejaculation, the most widely used definition is found in the *Diagnostic and statistical manual of mental disorders* (4th edition, DSM-IV). It includes: 1. the persistent or recurrent onset of orgasm and ejaculation with minimal sexual stimulation before, upon, or shortly after penetration and before the person wishes it. 2. marked distress or interpersonal difficulty. 3. premature ejaculation not due exclusively to the direct effects of a substance such as opiate withdrawal.

The DSM-IV also cautions that the physician should consider mediating factors such as age, novelty of situation or partner, and frequency of sexual activity. The DSM-IV diagnostic parameters include several features helpful in determining the severity of the problem: 1. onset or duration, whether lifelong or acquired (episodic) 2. context or range, generalized (global or all situations) or situational (some circumstances), and 3. etiology, due to psychological or combined) factors (psychological and medical or substance use (Metz *et al.*, 1997).

Some authors subdivide premature ejaculation into a primary form, which include individuals who had suffered from premature ejaculation since the beginning of their sexual lives, and into secondary form, men who developed the condition after years of satisfactory sexual functioning (e.g., Godpodinoff, 1989, Cooper *et al.*, 1993). Waldinger and coworkers (1997b) prefer an operational definition of premature ejaculation: an intravaginal ejaculation latency time of ≤ 1 minute in more than 90% of episodes of sexual intercourse, independent of age and duration of relationship.

While surveys among different populations vary considerably, epidemiological studies suggest that premature ejaculation is the most common

male sexual dysfunction (Metz *et al.*, 1997). Kinsey *et al.* (1948) conducted open interviews in the 1930s and 1940s in 5300 men and found a prevalence of 75%. However, premature ejaculation was defined as ejaculation occurring within 2 minutes after vaginal penetration, and the sample was non-representative for the whole population (Metz *et al.*, 1997). In a recent study of Laumann *et al.* (1994), a prevalence of 29% was found. This is in line with Althof (1995), who reports a prevalence of 30-40%.

TREATMENT OF PREMATURE EJACULATION

Local anaesthetics

Over the years, various local anaesthetic agents have been used in an attempt to delay ejaculation by reducing penile sensory input. These have become known as genital desensitizers and include dibucaine (Aycock, 1942), nupercaine (Schapiro, 1943), and benzocaine (Damrau, 1963). Nowadays, a 10 per cent lidocaine preparation is most commonly used.

Stop-start technique

Originally developed by Semans in the 1950s, and described in detail by Zilbergeld (1992) is a behavioral technique to develop ejaculatory control in men with premature ejaculation. Masters and Johnson (1966) devided the sexual cycle into four separate phases: 1. the excitement phase, 2. the plateau phase, 3. the orgasmic phase and 4. the resolution phase. Although this somewhat simple model has been subjected to criticism by various authors (e.g. Slob, 1994), it is still very useful as a model for the sexual response. The plateau phase can be subdivided in a control zone (Zilbergeld, 1992) and a point of ejaculatory inevitability (Masters and Johnson, 1966). Physiologically, this is the moment that secretions are activated and sperm is transported from the distal epididymis, *vasa deferentia*, seminal vesicles, and the prostate to the prostatic urethra (Lue, 1995). The control zone represents sexual arousal. The stop-start technique mainly focuses on training of awareness at what level this anxiety/arousal becomes the point of inevitability. If the patient with premature ejaculation feels that he is about to reach this point, he has to change his thrusting from faster to slower and maybe even stop for a moment to regain complete control. Progress in ejaculatory control should be made in a matter of weeks (Zilbergeld, 1992).

Squeeze technique

In addition to the stop-start technique, Masters and Johnson (1970) proposed the squeeze technique. The partners' thumb is put on the frenulum, and the first and second fingers are placed on the dorsal side of the penis to one another on either side of the coronal ridge. (Rather strong) pressure is applied by squeezing for an elapsed time of 3 to 4 seconds, and the male loses his urge to ejaculate.

Other techniques

Men have resorted to wearing multiple condoms, repeatedly masturbating prior to intercourse, not allowing partners to stimulate, or distracting themselves by performing complex mathematical computations while having sexual intercourse to overcome premature ejaculation. These tactics, however creative, curtail the pleasures of lovemaking and are generally unsuccessful (Althof, 1995).

It is now known that the impressive initial post-treatment success ranges from 60% to 95% are not necessary sustainable; 3 years after treatment, success rates dwindled to 25% (Althof, 1995). The existence of treatment failures, the relative lack of long-term gains of the existing treatment techniques, some reports on poor response to purely psychological treatment, the occasional rejection of psychological approaches, reports on pharmacological agents affecting various stages of sexual response cycle including ejaculation, and the pressures from third-party payers for succesful and cost-effective treatments all warrant exploration of new approaches to the treatment of premature ejaculation (Balon, 1996). In this chapter, a review is given of the most commonly used antidepressants and other psychoactive drugs in the treatment of premature ejaculation. In chapter 8, penile sensitivity threshold to vibrotactile stimulation was compared in men with premature ejaculation and/or erectile dysfunction and controls. In chapters 9 and 10, two prospective, double-blind, crossover placebo controlled studies are described with two serotonergic antidepressants, clomipramine and fluoxetine.

CLOMIPRAMINE

The tricyclic antidepressant clomipramine (Anafranil®) increases both ACTH and cortisol in humans (Golden *et al.*, 1989) and increases prolactin (Jones *et al.*, 1977, Golden *et al.*, 1989). It increases beta-endorphins in the rat hypothalamus

(Kurumaji *et al.*, 1986), and it has affinity for central dopamine-D_2, histamine-H_1, and alpha$_1$-adrenergic receptors (McTavish and Benfield, 1990). However, most importantly, it increases serotonin levels in the brain, through the indirect way of inhibition of serotonin (re)uptake inhibition, which results in increased levels of serotonin at postsynaptic receptors (McTavish and Benfield, 1990).

Clomipramine has been available for clinical use since 1966, and is now widely prescribed for the indications of depression, obsessive and compulsive disorders, anxiety, panic disorders and agoraphobia (McTavish and Benfield, 1990).

Clomipramine and sexual function

Sexual dysfunction due to clomipramine treatment in psychiatric patients is frequent and quite often severe (Crenshaw and Goldberg, 1996). In a multicentre study (520 patients), 41% of the men reported ejaculation failure (versus 2% in controls), 18% reported a lowering of libido (versus 7%) and 15% reported erectile dysfunction (versus 2%) (DeVeaugh-Geiss *et al.*, 1989). The average daily dose was 200 mg in this study. Other side effects noted in this report which could adversely affect sexual activity included fatigue, somnolence, constipation, dizziness, tremor, nausea, dyspepsia, dry mouth and nervousness (DeVeaugh-Geiss *et al.*, 1989). Another unusual side-effect of clomipramine was reported by McLean and coworkers (1983), who reported spontaneous orgasms in women when using clomipramine.

Only a few animal studies are known on the effects of clomipramine on sexual behaviors. Olivier and Mos (1988) found a decrease in number of ejaculations when male rats treated with clomipramine were behaviorally tested with an estrous female.

Clomipramine and premature ejaculation

In 1973, the English psychiatrist Eaton was the first to administer clomipramine to men with premature ejaculation in order to find out whether this would delay ejaculation in a group of nonpsychiatric patients. He noted benefit in 12 of 13 men. Improvement occurred within two weeks to two months on doses of 30 to 75 mg/day. While some patients required 75 mg/day for satisfactory effect, others could not ejaculate at all at this dose (Eaton, 1973). Surprisingly, it lasted until 1980 before other reports on the effect of clomipramine on premature ejaculation were published.

Goodman (1980) did a trial in 20 men with premature ejaculation. Four subjects (3 on clomipramine and 1 on placebo) dropped out of the study because of side effects. During four weeks, they received 10-40 mg clomipramine/day (or placebo), and during the next 4 weeks, all subjects were openly treated with clomipramine. No difference was noted between clomipramine and placebo over the first period, and subsequent improvement on clomipramine could have been placebo response. Unfortunately, the global evaluation did not define with precision to what extent premature ejaculation decreased. A follow-up after three months showed that 56% of the patients (9 of 16) still benefited from clomipramine. Two other patients reported a delayed ejaculation, but discontinued clomipramine due to side-effects (Goodman, 1980).

Another double-blind study published in 1980 by Porto, showed an improvement in 6 of 8 with clomipramine treated patients (30 mg/day) compared to none (0 of 10) on placebo.

The first double-blind crossover study was published in 1982 by Girgis and coworkers from Cairo University (Girgis *et al.*, 1982). Fifty patients were given either 20 mg/day of clomipramine or placebo for six weeks and then crossed over to the other drug condition for another 6 weeks. During the initial six weeks, 51% of the clomipramine-treated patients reported "satisfactory" intercourse, significantly different from the 32% in the placebo group. During the crossover, clomipramine patients, now on placebo, maintained their "satisfaction" rate (52%), while placebo patients switched to clomipramine improved significantly from 32% to 49% satisfactory intercourse. Again, as in the Goodman study (1980), the measurement of improvement was unfortunately inadequate. However, a important finding of the study of Girgis was, that a low dose of 20 mg/day clomipramine proved to be effective in improving ejaculatory satisfaction in patients with premature ejaculation.

The first report on clomipramine taken as needed for premature ejaculation, was done by Assalian in 1988. Two of the 5 patients that are described in these case studies, took clomipramine only when they anticipated sexual activity (25 mg, "at bedtime"). Of the 5 patients, continued improvement was noted by 2 patients on follow-up interviews 12 and 18 months later.

In 1993, Segraves and coworkers were the first to give a proper definition of premature ejaculation in their double-blind, placebo controlled study (Segraves *et al.*, 1993). It was defined as ejaculation within one minute of vaginal intromission or prior to eight penile vaginal thrusts. Average estimated time to ejaculation

after vaginal penetration increased from 51 seconds on placebo to 6.1 min with 25 mg/day clomipramine ($p<0.01$), and to 8.4 min on 50 mg/day clomipramine. Ratings of libido, erections, ejaculatory timing, ejaculatory quality, and overall sexual satisfaction all significantly improved with clomipramine, but none of these ratings changed with placebo. None of the 20 subjects dropped out of the study because of side-effects.

Another important factor in treatment of premature ejaculation was firstly described by Althof *et al* in 1995: the satisfaction of the female partner was included. They studied 15 couples in which men had lifelong (or primary) premature ejaculation. Men received placebo, 25 mg/day clomipramine and 50 mg/day placebo in random order and after a 2 month period, in which they did not receive any medication, a follow-up assessment was performed. The self-estimated mean time to ejaculation at base-line was 81 seconds. It increased to 202 seconds with 25 mg/day clomipramine and to 419 seconds with 50 mg/day. After the 2 months with no medication, it returned to base-line level. Three of 5 women who reported that they had never experienced an orgasm during intercourse became coitally orgasmic. Six of 10 women who previously had orgasms during sexual intercourse, reported that this occurred more frequently during clomipramine treatment (Althof *et al.*, 1995).

Although clomipramine proved to be an effective drug in the treatment of premature ejaculation, there were still a number of questions to be asked. Is clomipramine as effective in men with primary premature ejaculation as in men with (secondary) premature ejaculation and erectile dysfunction? What is the effect of clomipramine on sexual function in men without erectile and/or ejaculatory problems? What is the effect of clomipramine on erection, as (objectively) assessed in the psychophysiological laboratory? What is the effect of clomipramine on nocturnal penile tumescence? These and other questions were investigated and are described in chapter 9 of this thesis.

FLUOXETINE

Fluoxetine (Prozac®) is a selective serotonin (re)uptake inhibitor which is clinically used as an antidepressant (Benfield *et al.*, 1986), and for indication of obsessive-compulsive disorder and bulimia. The potency of inhibition of (re)uptake of radio labelled serotonin is similar to that of clomipramine, and

greater than that of imipramine and nortriptyline (Wong *et al.*, 1974, 1975). Although animal *in vitro* studies implied an inhibition for noradrenaline, this was not confirmed in humans *in vivo* (Bowsher *et al.*, 1985). Other (very) weak receptor affinities include alpha$_1$, alpha$_2$, beta, histamine, muscarinic, opoid, gamma-aminobutyric acid-benzodiazepine and dopamine receptors (Wong *et al.*, 1983). However, these have no clinical implications (Benfield *et al.*, 1986). When taken orally, it has a serum half-life of about 4 days, and its metabolite, desmethylfluoxetine, which is also a selective serotonin reuptake inhibitor, has a serum half-life of about 7 days (Benfield *et al.*, 1986).

Fluoxetine has been available for clinical use since the 1980's, and indications are depression (both unipolar and bipolar) and obsessive-compulsive disorders. The therapeutic range for psychiatric disorders is 20-80 mg/day, and it takes about 2 weeks after onset of medication, before the effect reaches a steady state (Benfield *et al.*, 1986).

Fluoxetine and sexual function

Numerous case reports on the effects of fluoxetine on sexual function in both females and males have been published since 1989. In that year, Kline reported about 2 cases: the first was a 35-year old woman who could not reach orgasm when using 20 mg/day fluoxetine for her depression and obsessive-compulsive disorder. This problem was partially resolved when she lowered the dose to 20 mg every other day. The other case was a 47-year old man using fluoxetine for depression. He reported difficulty attaining orgasm after 5 weeks of 40 mg/day. The retardation of orgasm was reduced by lowering the dose to 20 and 40 mg fluoxetine on alternate days.

Herman *et al.* (1990) reported 5 cases (3 men and 2 women). The 3 men developed orgasmic delay in the first week of their fluoxetine treatment (20 mg/day). In one case, this symptom resolved after 4 weeks without discontinuing fluoxetine treatment. The 2 women became anorgasmic when daily treated with 20-80 mg fluoxetine.

Musher (1990) reported that the predicted 1.9% sexual dysfunction (manufacturer's information), was an estimation far too low. In his group of 32 psychiatric patients, 16% (5 of 32, 4 women, 1 man) had specifically complained of anorgasmia, whatever the form of sexual stimulation used.

Zajecka and coworkers (1991) reported of 77 patients with fluoxetine treatment for depression. Six of the patients (7.8%, 5 women, 1 man) reported

spontaneously of anorgasia or delayed orgasm. The dose was 20-80 mg/day fluoxetine and the orgasmic dysfunction occurred within the first 6 weeks of treatment.

Genital anesthesia has been reported during fluoxetine treatment (King and Horowitz, 1993). A 37-year old woman, treated with 20 mg/day fluoxetine for depression, developed within 2 weeks of treatment a total lack of sensation in her vulva and vagina. It was medically confirmed with a needle-prick test. After 7 weeks, when she terminated the fluoxetine treatment because she had recovered from depression, genital sensation gradually returned to normal.

In a study of 60 depressed patients, Patterson (1993) found, when systematically questioned, 45 patients (75%) reported retarded or absent ejaculation, even though most were treated with only 20 mg/day.

Smith and Levitte (1993) reported return of sexual potency in 3 elderly men (64-95 years old) after the onset of fluoxetine treatment. The men had long-standing impotence and in two cases associated with medical problems well known as organic causes of impotence (one case with insulin dependent diabetes mellitus with peripheral neuropathy and vascular disease, and also post traumatic stress disorder and one case with Paget's disease, congestive heart failure and metastatic basal cell carcinoma).

Other case reports of sexual difficulties in depressive patients using fluoxetine include erectile difficulty and orgasmic delay (Neill 1991), retarded ejaculation that was not reduced with additive cyproheptadine (Feder 1991), lack of sensation in the penis, combined with orgasmic difficulty (Measom 1992), and delayed orgasm and prolonged erections (Swenson 1993). In these and above mentioned case studies, the orgasmic delay was always addressed as "negative side-effect", or "dysfunction." Also importantly to note, is that all cases described above were psychiatric patients, of whom it was not known if they had sexual dysfunctions before the onset of fluoxetine treatment. They were not asked systematically for sexual problems in prospective studies.

In 1994, the British psychiatrist Power-Smith was the first to call the ability to delay orgasm with fluoxetine a *beneficial* side-effect of fluoxetine, describing 2 cases of men with depression and erectile dysfunction, that disappeared with fluoxetine (20 mg/day). She was the first to suggest that fluoxetine could possibly be of any help in men with premature ejaculation and erectile dysfunction (Power-Smith, 1994).

Fluoxetine and premature ejaculation

Although reports on orgasmic delay with the use of fluoxetine had been available since 1989, and even since 1973 the use of serotonergic agents had been suggested for treatment of premature ejaculation (see above), it lasted until 1995 before the first study on the effects of fluoxetine on sexual function in men with premature ejaculation without psychiatric disorders was published.

In a study of 25 men with PE, Graziottin (1995) found that 20 men (80%) experienced a delay in ejaculation, although no further details were given. Three men accomplished a vaginal intromission for the first time in their lives when using fluoxetine (initially 20 mg/day, then lowered to 20 mg every other day).

The first double-blind study was reported by Kara and coworkers (1996). Nine men with premature ejaculation received daily 20-40 mg fluoxetine, and eight other men with premature ejaculation placebo. Three of the included subjects were diagnosed with depression (and therefore should be ruled out of the study). Two men discontinued the fluoxetine treatment because of side effects (nausea, headache and insomnia). Mean latency to ejaculation increased from 25 to 180 sec (fluoxetine, $n= 7$, $p<0.05$) and 30 to 60 sec (placebo, $n= 7$, n.s.).

In an open clinical trial in 11 patients with premature ejaculation Lee and coworkers (1996) found that 20-60 mg/day fluoxetine significantly increased median ejaculatory latency from less than 1 minute (pretreatment) to almost 10 minutes (fluoxetine treatment). Although this study was not placebo controlled, nor double-blind, these results are important, because for the first time the authors used a manner of measuring ejaculation latency: self-estimated time from vaginal intromission to ejaculation.

In chapter 10, a prospective, double-blind, placebo controlled study on the effect of fluoxetine in men with premature ejaculation, premature ejaculation and erectile dysfunction, erectile dysfunction alone and healthy controls is described.

OTHER SEROTONIN REUPTAKE INHIBITORS

Paroxetine

The novel selective serotonin reuptake inhibitor paroxetine (Seroxat®) is also used as an antidepressant and is more selective to 5-HT reuptake inhibition than fluoxetine. In a double-blind trial in patients with depression, Peselow *et al.* (1989) found that 13% claimed a retarded ejaculation, compared to 0% with

imipramine and placebo. In a similar trial, Claghorn (1992) reported a 17% retarded ejaculation rate on paroxetine versus 0% on placebo. Crenshaw and Goldberg (1996) claim a retarded or absent orgasm/ejaculation rate in about 50% of the patients.

In a double-blind trial in non-depressed patients with premature ejaculation, Waldinger *et al.* (1994) found that paroxetine caused the patients to have an increase in estimated intravaginal ejaculation latency time from a median of 30 sec (placebo) to 10 min (40 mg/day paroxetine). In an open trial, without placebo control, Ludovico and collaborators (1996) found an increase in ejaculation latency from less than 60 sec to "about" 15-20 min in a group of 32 men with PE.

In a recently published study of 34 patients, Waldinger *et al.* (1997a) found no difference between 20 mg/day and 40 mg/day paroxetine in increasing ejaculation latency in men with premature ejaculation. Baseline median values of estimated intravaginal ejaculation latency time (by patient and partner) was 12 seconds (range 3-45), this increased with 20 mg/day paroxetine for 8 weeks to a median of 300 seconds (range 10-1800), and with 40 mg/day for 8 weeks to a median of 540 (range 60-1200).

Fluvoxamine

Fluvoxamine (Fevarin®) is also a antidepressant with selective 5-HT reuptake inhibitory properties, but is less potent than the agents described above (e.g., Tulp *et al.*, 1991). Therefore, only few reports of the effects of fluvoxamine on ejaculatory behavior have been reported. Dorevitech and Davis (1994) reported of a 64-year old man treated with fluvoxamine for depression, who was unable to have an erection or ejaculation. He regained normal sexual function after terminating fluvoxamine treatment. Crenshaw and Goldberg (1996) reported that "about" 10-30% of the men and women that use fluvoxamine for panic disorders, phobias and depression complain of decreased libido and retarded orgasm.

MISCELLANEOUS PSYCHOTROPIC DRUGS AND EJACULATION

Monoamine oxidase inhibitors

The monoamine oxidase inhibitors (MAOIs) are mainly used in the treatment of neurotic or atypical depression. These drugs increase the levels of epinephrine, norepinephrine, dopamine and serotonin (Meston and Gorzalka, 1992) The

MAOIs have been known for their sexual side effects, with an incidence up to 20-40% (Harrison *et al.*, 1985). Delayed or inhibited ejaculation is reported for phenelzine (Harrison *et al.*, 1985), pargyline (Kohn, 1964) and tranylcypromine (Decastro, 1985). Bennet (1961) reported three cases of succesful treatment of premature ejaculation with isocarboxazid.

Cyproheptadine

Cyproheptadine is an antihistaminic, formerly used in Cushing's disease and anorexia nervosa. It also increases serotonin levels in the brain (Meston and Gorzalka, 1992, Rubin, 1993). Several reports indicate that cyproheptadine is able to convert drug-induced orgasmic failure in both men and women (e.g., Jeffries and Walker, 1987, Zajecka *et al.*, 1991). Decastro (1985) described a patient with no history of ejaculatory dysfunction suffering from monoamine oxidase inhibitor-induced inhibition of ejaculation. The disorder remitted when cyproheptadine was taken 1 hour prior to intercourse. Similarly, Arnott and Nutt (1994) reported the use of cyproheptadine two hours prior to intercourse to reverse anorgasmia in a 63-year old man treated for depression with the serotonin reuptake inhibitor fluvoxamine.

Yohimbine

The alpha$_2$ adrenoceptor antagonist yohimbine is known for is stimulating effects of male rat sexual behavior (Clark *et al.*, 1984). In humans yohimbine is used for its enhancement of penile erections in both men without sexual difficulties and men with erectile dysfunction, and enhancement of sexual desire in both women and men (Crenshaw and Goldberg, 1996). In a prospective, double-blind, placebo controlled crossover study, Rowland *et al.* (1997) found a strong positive effect on erection in 3 of 11 men with erectile dysfunction and an increase in sexual desire in all.

Price and Grunhaus (1990) reported a placebo-controlled case study in which yohimbine reversed the lack of ejaculation in a 35-year old man treated with clomipramine. Jacobsen (1991) treated 15 patients with fluoxetine-induced sexual dysfunction (6 women, 9 men) with yohimbine added to fluoxetine. Sexual dysfunction was partially or completely reversed in 11 of the 15 subjects. The beneficial effects of yohimbine in reversing fluoxetine-induced retarded ejaculation were confirmed in a double-blind study (Jacobsen, 1992).

Benzodiazepines

A number of benzodiazepines effective in treating generalized anxiety and panic attacks are also known to inhibit ejaculation in some men, presumably by enhancing gamma-aminobutyric acid (GABA; Metz *et al.*, 1997). These drugs include chlordiazepoxide (Hughes 1964, Buffum, 1992), lorazepam (Segraves, 1987), diazepam (Munjack and Oziel, 1980) and alprazolam (Hubbart *et al.*, 1991, Buffum, 1992). The effects on ejaculation, however, are not pervasive or as dramatic as that of the serotonin reuptake inhibitors, and controlled studies suggest that fewer than 10% of men experience an inhibition of ejaculation with these antianxiety medications (Metz *et al.*, 1997).

Antipsychotics

Amphetamine is a stimulating drug with affinity for different receptors in the central nervous system. It stimulates release of dopamine, inhibits monoamine oxidase and blocks the reuptake of both catecholamines and serotonin (Meston and Gorzalka, 1992). It is reported to delay ejaculation in subjects without ejaculatory dysfunction (Angrist and Gershon, 1976).

Cocaine is an addictive "recreational" drug and stimulates the central nervous system through blocking of monoamine neurotransmitters (Meston and Gorzalka, 1992). Different reports confirm that delayed ejaculation appears to be the most common sexual side-effect (e.g., Smith *et al.*, 1984, Cocores *et al.*, 1988)

Phenoxybenzamine

Phenoxybenzamine is an alpha-adrenergic blocking agent which is reported to have a suppressive action on ejaculation (Beretta *et al.*, 1986). This drug interferes with sympathetic nervous activation, and Shilon and coworkers (1984) found that phenoxybenzamine abolished the discharge of semen into the urethra and delayed ejaculation, although subjects continued to experience orgasm. Phenoxybenzamine has become a world-famous drug as an agent to initiate a penile erection. Virag (1982) was the first to report the possibility to inject another substance, papaverine, intracavernously to treat erectile difficulty. Brindley (1983) found the same effect with phenoxybenzamine and presented his discovery at the 1983 Annual Meeting of the American Urological Association in Las Vegas, Nevada. During his presentation, he wore a jogging outfit, rather than a business suit. He told the audience that, shortly before his talk, he had injected

phenoxybenzamine into his own penis. At that point, he lowered his pants and demonstrated his rigid erection (Crenshaw and Goldberg, 1996).

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Chapter 8

Penile sensitivity in men with premature ejaculation and erectile dysfunction*

PREMATURE EJACULATION is a common male dysfunction in which ejaculation/orgasm occurs before the individual desires due to the absence of voluntary control during sexual activity (DSM III-R, 1980). The etiology of premature ejaculation (PE) is poorly understood, although several theories regarding its origin and maintenance have received widespread attention (Ruff and StLawrence, 1985). The idea that low ejaculatory threshold results from infrequent sexual activity has received support from several studies (Masters and Johnson, 1970, Hastings, 1971, Spiess *et al.*, 1984). Cognitive-affective factors such as anxiety (Kaplan, 1981, Wolpe, 1982) and misperceived levels of sexual arousal (Kaplan, 1981) are also postulated to contribute to PE. Not surprisingly, a number of personality variables, including weakened self-image and hypomania, are associated with PE as well (Tondo *et al.*, 1991). As of yet, no single psychological or behavioral explanation for premature ejaculation has gained widespread acceptance, either because the proposed explanation has not been adequately tested or because it has not been consistently supported with empirical evidence.

Although the origin of premature ejaculation is often presumed to be psychological, a role of somatic factors cannot be ruled out. Ejaculation is a neural reflex stimulated by the sensory input to the penis and mediated by smooth and striated muscle contractions which produce seminal emission and expulsion (Kedia and Markland, 1975). Furthermore, the ejaculatory reflex is not dependent upon neural central neural control, as it remains intact in patients suffering from spinalectomy (Kedia and Markland, 1975, Szasz and Carpenter, 1989). It is, thus, conceivable that individual differences in the neurophysiological properties of penile receptors and effectors may partially

* Rowland DL, SM Haensel, JHM Blom, AK Slob. Penile sensitivity in men with premature ejaculation and erectile dysfunction. *J Sex Marit Ther* 1993, 19: 189-197.

account for variance in the ease and speed of the ejaculatory reflex. A role for somatic factors in the etiology of PE has, in fact, received some attention. Several reports have suggested that men with PE may be hypersensitive to physical stimulation (Damrav, 1963, Pasini, 1984, Strassberg *et al.*, 1990), although this idea has yet to be empirically tested. Recently, it has also been reported that men with PE exhibit decreased bulbocavernosal reflex latency to penile sensory stimulation (Godpodinoff, 1989). As with current psychological theories, it is unknown whether such physiological parameters represent consequences or causes of the PE.

The present study sought to elucidate further the role of somatic factors in ejaculation latency. Specifically, we examined whether penile sensitivity to physical stimulation might distinguish premature ejaculators from sexually functional men. Consistent with the idea of hypersensitivity in men with PE (Damrav, 1963, Pasini, 1984), sensory thresholds in such men might be lower than in men with normal ejaculatory latency. In this study, subjective threshold to vibrotactile stimulation of the penis was established in four groups, two of which included men who were diagnosed as having premature ejaculation. One of these groups was characterized by PE that was both exclusive and long-term, while the other showed late-developing PE in combination with erectile dysfunction. For purposes of comparison, two additional groups of men indicating no ejaculatory dysfunction were included: a sexually functional group, and an erectile dysfunction group.

METHOD

Subjects

Sixty-three men participated in this study. Of these, 33 were healthy, sexually functional controls (group = Contr) recruited through word-of-mouth. These men were first interviewed extensively regarding their sexual functioning and then asked to provide further information, including a detailed medical and sexual history, through questionnaire responses on a sexual functioning inventory. Subjects were screened for any medical disorders and treatments, including medications, that might affect sexual functioning, and more specifically, ejaculation latency. None of these control men indicated any difficulty with achieving or maintaining an erection, none indicated having

premature ejaculation, and all reported ejaculation latencies following vaginal intromission of four minutes or more when desired. All men indicated exclusive heterosexual preference. All but one were either married, living with their sexual partner, or in a steady sexual relationship. Subjects indicating an ongoing relationship had been with their sexual partner for an average of 11.2 years (range = 1-30).

An additional 30 men were drawn from the Urology Clinic of the Erasmus University Academic Hospital, Dijkzigt. These men had approached the clinic

TABLE 8.1. Demographic and sexual characteristics of sexually functional and dysfunctional groups

Item	Controls	PE	PE/ED	ED
Demographics				
Number of subjects	33	9	8	13
Age (years)	38.2	47.7	49.1	49.1
Percent with sex partner	96.9	77.7	75.0	76.9
Mean length of relationship (yr)	11.2	14.8	26.0#	20.5#
Mean length of sexual dysfunction (yr)	n.a.	14.9	4.9	6.8
Quality of life[1]	5.3	3.3#	3.1#	2.8#
Erectile function[2]				
Problem getting erection	1.5	2.6	4.4#	5.8#
Problem getting erection before coitus	2.7	2.3	5.9#	5.8#
Problem keeping erection	1.8	4.9#	5.9#	6.8#
Problem keeping erection before coitus	2.7	3.9	7.0#	6.2#
Problem keeping erection during coitus	1.4	5.5#	6.3#	6.5#
Ejaculatory function[2]				
Problem ejaculating	1.3	1.7	1.3	2.2
Ejaculation during coitus	6.9	6.6	6.4	4.4
Ejaculation when partner stimulates penis	5.3	6.3#	5.0	4.9
Ejaculation quicker than desired	2.2	6.8#	6.7#	2.7
Duration of coitus to ejaculation[3] (min)	7-10	0-2#	0.5-2#	5-7

[1] For this item, 1= very poor, 7= excellent
[2] 1= not at all, 7= always
[3] represents median interval
# Indicates $p<0.05$ (t-test or Mann-Whitney) compared with controls

for one or both of two dysfunctions, erectile problems or premature ejaculation, and were selected for study upon meeting the criteria indicated below. All were exclusively heterosexual and 23 were involved in an ongoing sexual relationship. For these subjects the average length of their relationship was 20.7 years (range = 1-38).

As part of a routine diagnostic procedure, these patients underwent an extensive interview on their sexual and medical history, and a complete physical examination, which included Doppler investigation of the cavernous arteries, penile/brachial index measurement, and a neurological examination. For all

patients with erectile dysfunction, papaverine injection was also included. If papaverine injection did not produce sufficient erection, patients underwent further investigation, which could include duplex scanning, dynamic cavernosography, or angiography. Routine endocrino-logical analysis was carried out for all patients to ensure that they fell within the normal range for testosterone, luteinizing hormone, and prolactin. Patients on medication known to interfere with erectile or ejaculatory response were not included in the study.

Based upon the presenting complaint of the patient, medical and sexual records from the Urology Clinic, an interview with the clinician, and responses on a sexual functioning inventory, patients were assigned to one of three groups. The first group (n=9) consisted of patients with long-term (mean=14.9 years) premature ejaculation (PE). This group reported ejaculation latencies of 0-2 minutes following intromission but showed no evidence of erectile dysfunction. The second group (n=8) included patients having erectile problems, most often in *maintaining* an erection rather than achieving one, and premature ejaculation (PE/ED). This group also reported ejaculation latencies in the range of 0.5-2 min. Though not necessarily characteristic of all subjects in this group, five were known to have developed erectile dysfunction prior to premature ejaculation. Erectile dysfunction had endured for 4.9 years (mean) in this group. The third group of patients (n=13) was characterized by erectile dysfunction only (ED), with the major difficulty achieving erection (mean duration=6.8 years). These patients indicated no problems with premature ejaculation, although six reported low frequency of ejaculation caused by lack of intercourse due to erectile problems. Further characterization of each group is provided in table 8.1.

Apparatus and testing

Vibratory tactile stimulation to the penis was administered with a biothesiometer, modified with variable amplitude and adjustments to enable testing on the penis while the subject sat in an upright position (figure 8.1). The manner in which the stimulating surface of the vibrator was maintained at a constant pressure on the penis has been described elsewhere (Rowland and Slob, 1992). The surface area stimulated was 1.5 x 2.0 cm, the stimulus frequency was 120 Hz with a duration of 1 sec. A preliminary threshold was established using the psychological method of limits, in which stimuli were presented in ascending and descending order. Subsequently, the method of constant stimuli was used, in which stimuli of varying magnitudes were presented randomly. The actual

threshold was calculated as the average of at least five threshold crossings using this latter method of stimulation.

All subjects were stimulated at approximately the same location on the ventral surface of the flaccid penis, near the tip immediately posterior to the coronal ridge. The foreskin was not retracted for these tests.

Procedure

Before obtaining informed consent as approved by the University Hospital Medical Ethics Committee, subjects were instructed about all procedures involving the testing. For patients, this testing was often included as part of a larger overall assessment of sexual functioning, and they were informed that they were free to refuse or terminate testing at any time, as were all subjects in the study. Subjects were then asked to provide, both through personal interview and questionnaires, information pertinent to their sexual functioning. In

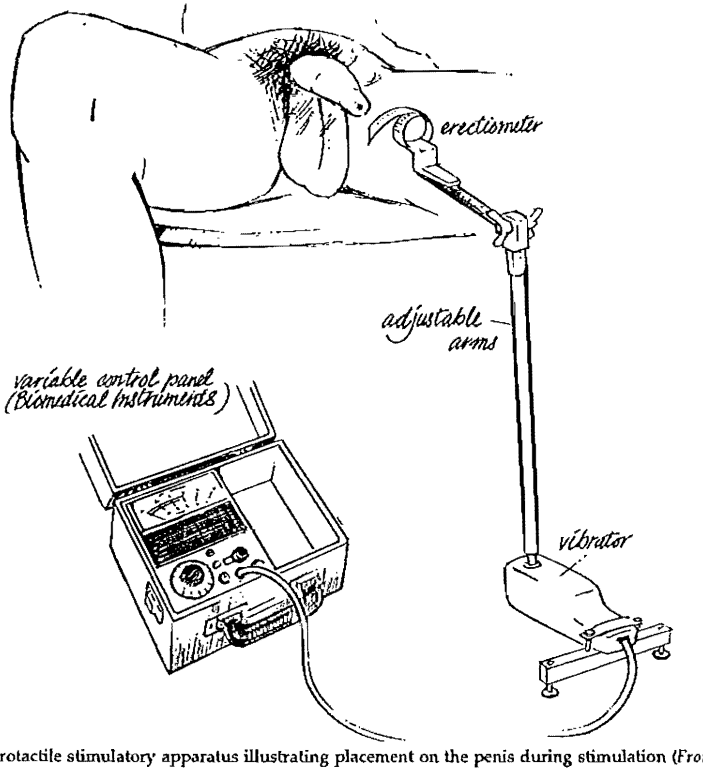

FIGURE 8.1. Vibrotactile stimulatory apparatus illustrating placement on the penis during stimulation (*From*: Rowland and Slob, 1992)

addition, medical histories and diagnostic information were obtained on patients from the Urology Clinic prior to the interview, so subjects could be questioned thoroughly and accurately regarding their sexual dysfunction.

During laboratory testing, the subject, nude from waist down, was seated in a sound-attenuated, dimly lit room to facilitate stimulus detection. After securing the vibrator to the subject's penis, the experimenter retreated to an anteroom to commence testing. Subjects were tested in complete privacy with contact maintained only through an intercom.

Data analysis

Comparison of thresholds among groups was carried out using one-way analysis of variance with age as a covariate. Data were log transformed to satisfy homogeneity of variance requirements. Correlations were performed using the Pearson test. For all such tests the effect of age was partialled out as this variable is known to affect threshold (Rowland *et al.*, 1989), and depending on the number of control variables, either first- or second-order correlation values are reported. All probability levels represent two-tailed values.

RESULTS

Description of sexual functioning in controls and patients

Differences in the sexual functioning of the four groups are presented in table 8.1. As might be expected, men in the PE group showed little or no erectile problems, but did report short latencies to ejaculation. ED men showed significantly lower ratings on a number of measures of erectile functioning, including getting an erection. PE/ED men indicated primary difficulty *maintaining* erection and short latencies to ejaculation, but in general they did not indicate as much difficulty *getting* an erection.

Differences in penile sensitivity among groups

Figure 8.2 illustrates penile tresholds in each of the four groups of men. The Contr and PE groups showed low tresholds whereas the PE/ED and particularly, ED groups exhibit high tresholds. To determine whether differences existed among four groups, analysis of variance was performed using age as the

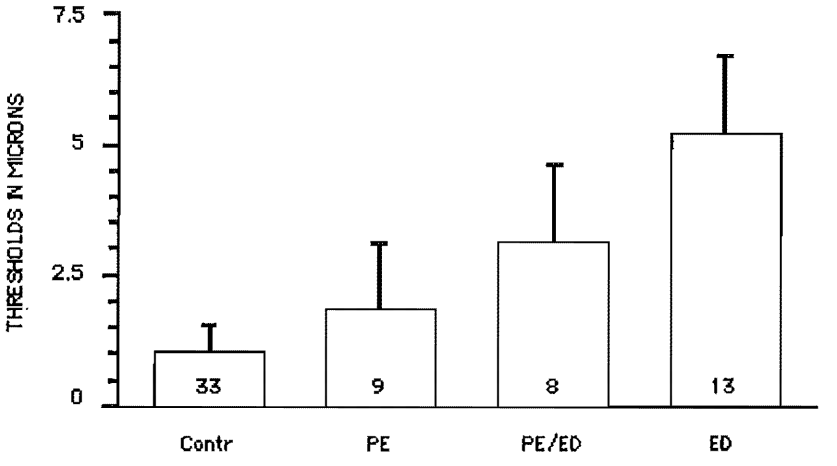

FIGURE 8.2. Mean (±SE) penile thresholds (microns of stimulus movement) to vibrotactile stimulation for men with premature ejaculation (PE), premature ejaculation and erectile dysfunction (PE/ED), erectile dysfunction (ED), and control subjects (Contr). Also indicated is the number of subjects per group.

covariate (age is known to correlate with penile sensitivity; Rowland *et al.*, 1989), with a resulting F value of 3.68, p=0.017. Individual comparisons (also with age as a covariate) indicated that control and PE groups did not differ from each other, but these groups did differ significantly from the ED group ($p<0.05$). Furthermore, the Contr group showed a marginal difference from the PE/ED group ($p<0.10$).

Pearson correlations between penile thresholds and measure of self-reported sexual functioning

Correlations of theoretical and statistical significance were found between penile sensitivity and self-reported sexual functioning (see table 8.2). Since thresholds were correlated with age, the variance contributed by this factor was partialled out for all correlations. Also, since frequency measures of various sexual activities are known to correlate with self-reported 'opportunity for sex,' this factor also partialled out for those correlations.

When all groups were reviewed together, penile threshold correlated significantly with several erectile measures, such that higher tresholds were positively related to greater difficulty in getting (r=0.30; p=0.019) and holding (r=0.42; p=0.001) an erection, and negatively related to frequency of morning erections (r=-0.34; p=0.007) (see table 8.2).

TABLE 8.2. Pearson correlations between penile threshold and self-reported measures of sexual functioning[1]

Group	Item[2]	r	p
All groups	Frequency of morning erections	-0.34	0.007
	Quality of life	-0.31	0.011
	Problem of getting erection before coitus	0.30	0.019
	Problem keeping an erection before coitus	0.39	0.002
	Problem keeping erection during coitus	0.42	0.001
PE	Likelihood of orgasm	-0.66	0.068
	Duration of intercourse to ejaculation	0.69	0.039
ED	Frequency of morning erection	-0.71	0.006
	Problem getting an erection	0.57	0.043
	Overall enjoyment of sex	-0.67	0.012

[1] All probabilities represent two-tailed values
[2] For all items: 1= low, 6 or 7= high

When correlations were limited to specific groups, several patterns emerged. For the PE group, there was a strong correlation between self-reported ejaculation latency and threshold, with low thresholds related to short latencies ($r=0.69$; $p=0.039$). For the ED group, thresholds correlated with frequency/difficulty of several self-reported measures of erections within several contexts and with overall enjoyment of sex ($r=0.67$; $p=0.012$).

DISCUSSION

To our knowledge, this is the first report to indicate that subjective threshold to vibrotactile stimulation of the penis in men with premature ejaculation does not differ from that of sexually functional men. As such, these data suggest that differences in ejaculation latency between functional men and premature ejaculators cannot be attributed to hypersensitivity of surface receptors of the penis. Yet, while receptor hypersensivity is not characteristic of PE men, the contribution of receptor sensitivity cannot be ruled out entirely. For one, this study does not provide some evidence that despite the normal range of thresholds for men with PE, ejaculation latency is related to penile sensitivity in these men. Second, these results do not preclude the involvement of deep (vs. surface) penile receptors in ejaculation latency, which may differ in sensitivity between control and PE men. Third, the possibility exists that during erection, receptor sensitivity greatly increases in men with PE. Although not tested in this

study, the third possibility is unlikely given the consistent pattern of decreased sensitivity during erection found in both sexually functional men and men with erectile dysfunction (Edwards and Husted, 1976, Rowland *et al.*, 1991).

Since penile dermal sensitivity does not appear to differentiate PE men from controls, variation in speed and ease of ejaculation most likely arises from other components of the sexual response. It may be that for some individuals, the motor component of the ejaculatory reflex arc is more readily triggered by sensory stimulation (e.g., bulbocavernosal reflex; Godpodinoff, 1989). Or, despite a lack of strong evidence (Spiess et al., 1984, LoPiccolo and Stock, 1986), cognitive-affective factors may play the more salient role in this dysfunction. Although the ejaculation reflex can occur independent of central neural control, cognitive variables undoubtedly play a role in ejaculation latency by mediating levels of arousal to various contextual stimuli.

Men having symptoms of both premature ejaculation and erectile dysfunction (group PE/ED) exhibited decreased sensitivity relative to controls and PE men. Thresholds for the men in this group fell approximately midway between the PE and ED groups. In this respect, the low sensitivity of the PE/ED men may be related to their erectile dysfunction, as previous research has indicated high tresholds among such men (Edwards and Husted, 1976, Rowland *et al.*, 1991, Padma-Nathan *et al.*, 1986). Furthermore, the premature ejaculation that characterizes this group may well be secondary to the erectile dysfunction, that is, a number of these men admitted the possibility that their rapid ejaculation might have been related to their concern about losing their erection. The findings from the PE/ED group, which clearly indicate that premature ejaculation can occur in men with *decreased* penile sensitivity, argue strongly for a role for central (cognitive) factors in the mediation of ejaculation latency.

The etiology and classification of premature ejaculation suggest that there may be a number of different causes underlying this dysfunction. But penile dermal hypersensitivity does not appear to be a major factor contributing to short ejaculation latencies in the sample of PE men studied here. In addition, lowered sensitivity associated with erectile dysfunction does not prevent the occurrence of PE. Further study aimed at differentiating somatic from cognitive/affective contributions in men with similar somatic etiologies of premature ejaculation awaits implementation.

SUMMARY

Previous research indicates that penile sensitivity is typically lower in men with erectile dysfunction than in age-matched controls. On the assumption that sensitivity might be greater in men with premature ejaculation, the present research investigated penile threshold (sensitivity) to vibrotactile stimulation in men with premature ejaculation, erectile dysfunction, or a combination of the two. Premature ejaculators showed thresholds commensurate with controls, while men with erectile dysfunction, or combined erectile dysfunction and premature ejaculation, showed significantly elevated thresholds. Although premature ejaculators did not show penile hypersensivity, there was a significant correlation in this group between ejaculation latency and threshold. Overall, these findings argue against a primary role for penile sensitivity in ejaculation latency, and suggest that other somatic or cognitive factors may play the more critical role in premature ejaculation.

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Chapter 9

Clomipramine and sexual function in men with premature ejaculation and controls*

PREMATURE OR RAPID EJACULATION is a common male sexual dysfunction in which ejaculation and orgasm occur before desired due to lack of control during sexual activity (Althof, 1995a, 1995b, Althof *et al.*, 1995, Rosen, 1995). Although ejaculation latency undoubtedly is affected by psychological and cognitive mechanisms (Althof 1995a, chapter 8), somatic factors are also involved. Specifically, ejaculation is mediated partly though a neural reflex stimulated by sensory input to the penis, and terminating in smooth and striated muscle contractions that produce seminal emission and expulsion.

The primary therapeutic approach to premature ejaculation typically has been behavioral, and pharmacotherapy may best be reserved for cases when the behavioral approach has failed, or when mitigating factors make such treatment difficult or undesirable (Riley and Riley, 1993, Rosen and Ashton, 1993). However, the reported success of pharmacotherapeutic treatment in enabling ejaculatory control has focused greater attention on alternatives to behavior therapy, particularly on pharmacological agents purported to mediate this effect. An agent that has been used successfully to treat premature ejaculation is clomipramine, a tricyclic antidepressant and serotonin reuptake inhibitor (Assalian, 1988, Bech, 1988, Riley and Riley, 1993, Segraves *et al.*, 1993, Althof, 1995a, Althof *et al.*, 1995, Rosen, 1995).

We attempted to determine the efficacy of clomipramine given as needed under rigorous testing conditions using a prospective, randomized, double-blind, placebo controlled crossover design. A group of sexually functional volunteers was included for comparison. Unlike earlier studies that have relied on simple global self-assessment of sexual function, we used several tests to assess various

* Haensel SM, DL Rowland, KTHK Kallan, AK Slob. Clomipramine and sexual function in men with premature ejaculation and controls. *J Urol* 1996, 156: 1310-1315. Presented at the 91st Annual Meeting of the American Urological Association, Orlando, Florida, May 1996, and at the XLVII Kongreß der Deutschen Gesellschaft für Urologie e.V, Düsseldorf, October 1996.

components of the sexual response cycle, including sexual desire, sexual and erectile arousal, and ejaculation. Since ejaculation was the primary dependent variable of interest, it was measured further with multiple indexes, including self-assessment of ejaculatory response through daily logs, overall assessment at the end of each treatment phase, and laboratory measurement of erectile, arousal, and ejaculatory response to visual erotic and penile tactile stimulation (Rowland and Slob, 1992, 1995, Rowland *et al.*, 1994, Janssen *et al.*, 1994, chapter 8).

METHOD

Subjects

The experimental group consisted of 14 patients, recruited from the urology outpatient clinic of a university hospital, who satisfied the DSM IV (*Diagnostic and statistical manual of mental disorders*, 4th edition) criteria for premature ejaculation. Eight patients 31 to 61 years old (mean age 42.2) had primary premature ejaculation and 6 patients 26 to 52 years old (mean age 41.2) had secondary premature ejaculation concomitant with erectile dysfunction (Cooper *et al.*, 1993). Erectile dysfunction was defined as the inability to achieve or maintain an erection sufficient for sexual intercourse. Patients fulfilled the criteria of age 18 years or older, heterosexual orientation, sexual dysfunction at least 6 months in duration, premature ejaculation during coitus or masturbation, defined by self-reported inability to control ejaculation with significant duress, willingness to attempt intercourse or masturbation at least once a week, consent of sexual partner when appropriate, and no concomitant psychiatric (e.g., depression) or somatic disease, previous surgery or drug use known to affect sexual function. An age matched (mean age 40.5, range 26 to 52 years) group of 8 healthy, sexually functional volunteers with no sexual problems was recruited, mainly through word of mouth.

Study design

Before implementation of the prospective, randomized, double-blind, placebo controlled, crossover study, a pretest session was provided for initial evaluation and collection of data, and gave subjects the opportunity to adapt to the laboratory setting and procedures. Treatment phases consisted of two 3-week administration periods of placebo or clomipramine capsules. After the initial 3-

week phase, subjects underwent an additional 3-week crossover treatment, with placebo for those previously receiving clomipramine and clomipramine for those previously receiving placebo. Capsules of clomipramine and placebo appeared identical, and order of treatment (drug versus placebo) for the subjects was determined by the pharmacy department of the university hospital. Subjects self-administered the capsule (25 mg clomipramine or placebo) 12 to 24 hours before anticipated sexual activity (coitus or masturbation), and not more than twice a week.

Following completion of the study, the unblinded code revealed that 8 patients began with clomipramine and 6 with placebo. Five controls began with clomipramine and 3 with placebo.

During the pretest phase and at the end of each treatment phase (clomipramine and placebo), a series of self-assessments and objective measurements of sexual function were done. Self-assessment, based on extensive structured interview with a clinician, relied on a inventory of sexual function consisting of 56 items dealing with quantitative and qualitative aspects of the sexual relationship and sexual activities, and incidence of sexual problems (Slob *et al.*, 1990, Rowland *et al.*, 1994, chapter 8). As part of this questionnaire the various dimensions of sexual desire, arousal (including erectile function) and ejaculatory control were evaluated on 7-point scales.

Nocturnal penile tumescence

Objective assessment of genital response was achieved with nocturnal penile tumescence and waking erectile assessment procedures. Specifically, nocturnal penile tumescence data (increase in penile circumference) were collected during 4 consecutive nights with Erectiometers (figure 9.1; ESKA, Sommerville, New Jersey), consisting of calibrated 19 x 2 cm felt bands with plastic sliding collars used to measure penile tumescence. The yellow collar (used for 2 nights) moved with a strain of 250 gm and the green collar (used for 2 nights) moved with 450 gm. Thus, the

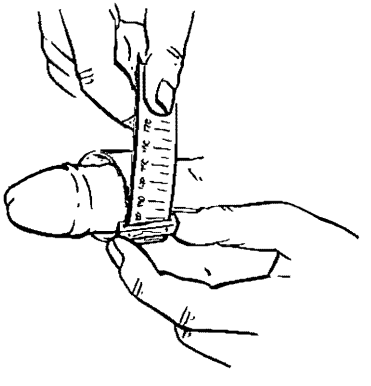

FIGURE 9.1. Erectiometer (see text)

Erectiometer provides information about rigidity and increase in circumference of the erect penis (Slob *et al.*, 1990).

Waking erectile assessment

In the laboratory, waking erectile assessment with visual sexual stimulation and penile tactile stimulation was performed (figure 9.2) using penile measurements (a yellow Erectiometer with attached mini-vibrator (12) around the tip of the penis was used to measure increase in penile circumference), and

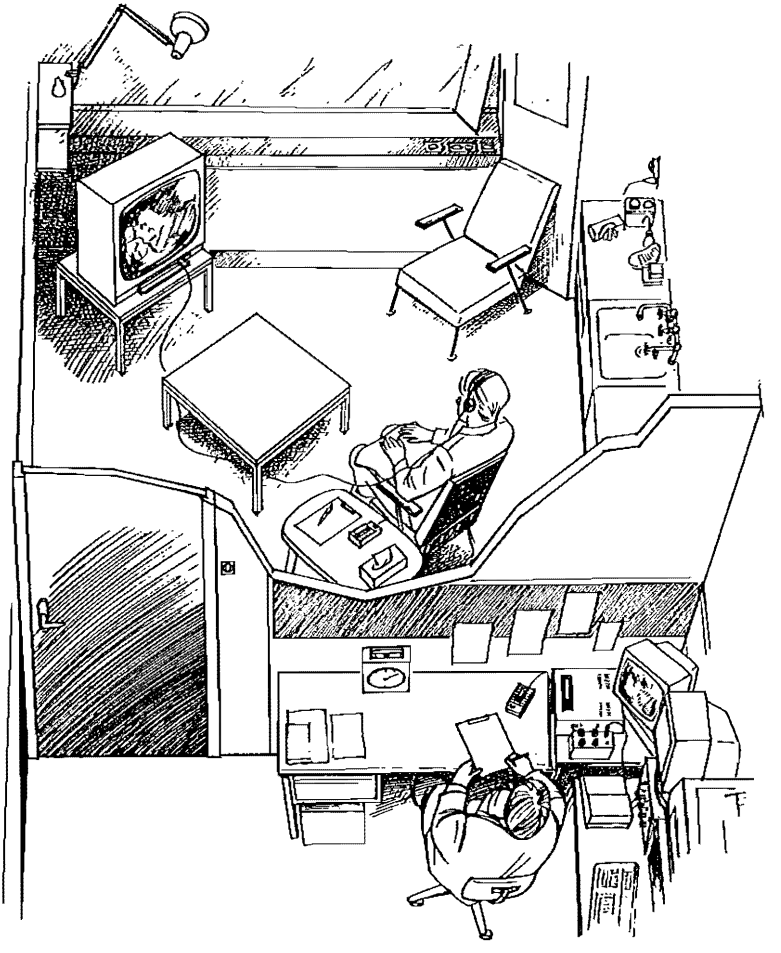

FIGURE 9.2. The psychophysiological laboratory (see text)

subjective assessment of arousal and genital response. The Erectiometer was positioned by the subjects, and subsequently inspected and read by the experimenter. The lap of the subject was covered to prevent visual genital feedback. Two different videos approximately 9 minutes long were used in each session, and different sets of videos were used in the different sessions (pretest and 2 drug phases). Because the videos used during treatment periods were always presented in the same order, they were counterbalanced across placebo and drug conditions. The videos were similar in types of explicit heterosexual activities portrayed.

Each subject was first exposed to an erotic video without and then with concomitant vibration to the penis. This order of presentation was selected because previous research indicated greater arousal and genital response to the combination of the video and penile vibration (Rowland *et al.*, 1994). Thus, the likelihood of ejaculation during the initial stimulus session (video) was minimized. Following each video, the subjects indicated whether they had ejaculated, and completed a number of self-report items assessing sexual, genital and ejaculatory response during the stimulus period. During the treatment phases, subjects were asked to take a capsule (clomipramine or placebo) 12 to 24 hours before the planned visit to the waking erectile assessment laboratory. During each treatment phase, subjects also completed short daily logs about various sexual activities, with particular attention paid to ejaculatory response. These logs were mailed once a week to the investigators. One control subject did not report daily sexual activities and, therefore, only waking erectile assessment laboratory measurements were included in the study.

Statistics

Data collected before treatment were analyzed with a 1-way analysis of variance to assess differences among groups except for waking erectile assessment analysis, which included a second factor of type of stimulation (erotic video or erotic video plus penile tactile stimulation). During the 2 treatment phases, 2-way analysis of variance for a mixed factorial design with 1 between factor (group) and 1 repeated measure (clomipramine versus placebo) was used to determine effects on measurements of sexual function drawn from 3 sources (daily logs, retrospective self-assessment at the end of each treatment phase and nocturnal penile tumescence). Because waking erectile assessment sessions

included a third variable, type of stimulation, a 3-way analysis of variance was used.

RESULTS

Pretreatment data

Table 9.1 indicates differences in sexual history and self-report measurements of sexual function before treatment between dysfunctional and functional groups. Briefly, there were striking differences regarding self-assessment items pertaining to sexual function, particularly in orgasm and ejaculation measurements. The dysfunctional men almost always reported having an orgasm sooner than desired ($p<0.001$), with fewer than 10 pelvic thrusts ($p=0.002$) and a shorter duration of intercourse (approximately 1 to 2 minutes, $p<0.001$) than men in the comparison group. With respect to other sexual difficulties, men with premature ejaculation combined with erectile dysfunction had greater difficulty keeping an erection ($p=0.031$) and fewer morning erections ($p=0.001$) than those

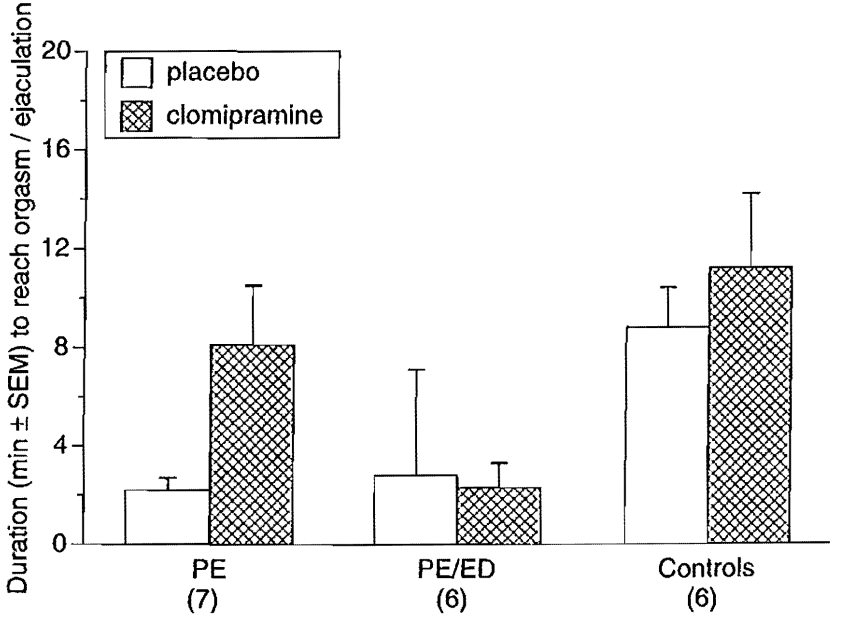

FIGURE 9.3. Mean (± SE) of estimated duration to reach orgasm or ejaculation with sexual activity at home, when clomipramine (25 mg) or placebo was taken 12 to 24 h earlier (double-blind, crossover design). PE: 7 men with premature ejaculation, PE/ED: 6 men with premature ejaculation and erectile dysfunction, Contr: 6 volunteers without sexual dysfunction

TABLE 9.1. Questionnaire and nocturnal penile tumescence (NPT) data (means ± SE) before and during clomipramine and placebo treatment. PE: premature ejaculation, PE/ED: premature ejaculation and ED, Contr: healthy volunteers without sexual dysfunction.

Item and description	Pretest mean ± SE	Statistics[1] F(2/18), p	No. Pts.	clomipramine mean ± SE	placebo mean ± SE	Statistics[2] F-value	p
Relationship score[3]							
PE	6.2 ± 0.4	3.0, 0.076	7	6.6 ± 0.2	5.4 ± 0.4	dr: F(1/17)=10.3	0.005
PE/ED	4.3 ± 0.7		6	4.7 ± 0.8	4.3 ± 0.8	gr: F(2/17)=2.9	0.0084
Contr	5.3 ± 0.6		7	6.1 ± 0.1	5.7 ± 0.5	dr x gr: n.s.	
Current sex life score[4]							
PE	3.6 ± 0.4	3.0, 0.075	8	5.0 ± 0.5	3.8 ± 0.2	dr: n.s.	
PE/ED	3.2 ± 0.5		6	3.3 ± 0.6	3.5 ± 0.2	gr: F(2/18)=9.2	0.002
Contr	4.6 ± 0.3		7	5.3 ± 0.3	5.3 ± 0.2	dr x gr: F(2/18)=2.6	0.10
Frequencies/week							
Morning erections							
PE	5.4 ± 0.6	16.3, <0.001	8	4.8 ± 0.8	5.0 ± 0.6	dr: n.s.	
PE/ED	1.2 ± 0.5		6	1.5 ± 0.7	1.7 ± 0.6	gr: F(2/18)=6.1	0.009
Contr	3.0 ± 0.5		7	4.3 ± 0.7	4.4 ± 0.9	dr x gr: n.s.	
Intercourse							
PE	2.2 ± 0.6		8	1.6 ± 0.5	1.9 ± 0.5	dr: n.s.	
PE/ED	1.3 ± 0.4		6	1.6 ± 0.4	1.7 ± 0.4	gr: n.s.	
Contr	1.4 ± 0.3		7	2.5 ± 0.4	1.6 ± 0.4		
Nocturnal penile tumescence[5] (mm)							
Yellow collar Erectiometer:							
PE	23.7 ± 2.2		7	24.6 ± 3.7	27.9 ± 1.0	dr: F(1/14)=7.8	0.014
PE/ED	18.0 ± 1.8		3	12.2 ± 4.0	17.5 ± 3.8	gr: F(2/14)=3.7	0.051
Contr	26.0 ± 2.9		7	24.4 ± 3.9	31.6 ± 3.3	dr x gr: n.s.	
Green collar Erectiometer:							
PE	19.1 ± 2.7		7	14.4 ± 3.7	23.4 ± 3.9	dr: F(1/14)=18.1	<0.001
PE/ED	16.2 ± 5.0		3	10.5 ± 2.5	14.2 ± 3.3	gr: n.s.	
Contr	22.7 ± 4.5		7	19.4 ± 2.4	28.2 ± 3.4	dr x gr: n.s.	
Sexual difficulties score[6]							
Getting an erection when wanted							
PE	2.0 ± 0.7		8	2.8 ± 0.9	2.1 ± 0.4	dr: n.s.	
PE/ED	3.3 ± 0.7		6	4.3 ± 0.9	3.5 ± 0.8	gr: F(2/18)=3.5	0.025
Contr	1.6 ± 0.6		7	1.9 ± 0.7	4.2 ± 0.9	dr x gr: n.s.	
Keeping an erection when wanted							
PE	2.6 ± 0.8	4.2, 0.031	8	2.8 ± 0.8	3.5 ± 0.6	dr: n.s.	
PE/ED	5.3 ± 1.0		6	5.2 ± 1.0	4.2 ± 0.9	gr: F(2/18)=4.6	0.024
Contr	2.1 ± 0.5		7	1.8 ± 0.7	1.7 ± 0.3	dr x gr: n.s.	
Fear of failure							
PE	2.8 ± 1.0		8	4.4 ± 1.0	3.6 ± 1.0	dr: n.s.	
PE/ED	3.3 ± 1.1		6	4.2 ± 1.3	4.3 ± 1.1	gr: n.s.	
Contr	5.6 ± 0.8		7	5.9 ± 0.6	5.9 ± 0.6		
Orgasm/ejaculation sooner than wanted							
PE	1.0 ± 0.0	45.9, <0.001	8	4.0 ± 0.8	1.1 ± 0.1	dr: F(1/18)=8.0	0.01
PE/ED	1.2 ± 0.2		6	1.8 ± 0.5	1.8 ± 0.8	gr: F(2/18)=14.3	<0.001
Contr	5.3 ± 0.6		7	5.6 ± 0.6	5.7 ± 0.5	dr x gr: F(2/18)=7.5	0.04
Intercourse until orgasm/ejaculation							
Duration (min)							
PE	1.4 ± 0.3	36.5, <0.001	6	2.8 ± 0.6	1.7 ± 0.3	dr: n.s.	
PE/ED	1.0 ± 0.0		5	1.2 ± 0.2	1.2 ± 0.2	gr: F(2/13)=18.7	<0.001
Contr	3.7 ± 0.3		5	3.6 ± 0.2	3.8 ± 0.2	dr x gr: F(2/13)=2.9	0.09
Number of pelvic thrusts							
PE	1.9 ± 0.4	8.7, 0.002	6	4.0 ± 0.0	3.3 ± 0.5	dr: n.s.	
PE/ED	1.5 ± 0.5		5	1.8 ± 0.4	1.5 ± 0.4	gr: F(2/14)=21.0	<0.001
Contr	3.7 ± 0.3		6	3.7 ± 0.3	4.0 ± 0.0	dr x gr: n.s.	

Judgements are shown for preceding 3-week period. NPT data were collected during 4 nights before lab visit. [1] 1-way analysis of variance. [2] 2-way analysis of variance, factors drugs (dr) and groups (gr). [3] 1-very unhappy, 4-neutral, 7-very happy. [4] 1-very bad, 4-neutral, 7-excellent. [5] mean increase in circumference for 2 nights. [6] 1-always a problem, 3-50% of the time, 5-never a problem. [7] 1-always, 3-50% of the time, 5-never

TABLE 9.2. Psychophysiological data obtained in the laboratory during visual erotic stimulation without and with concomitant vibrotactile stimulation to the penis. PE: primary premature ejaculation, PE/ED: premature ejaculation secondary to ED, Contr: healthy comparison group.

Item and description	Pretest mean ± SE	Statistics[1] F, p-values	placebo mean ± SE	clomipramine mean ± SE	Statistics[2] F, p-value
How sexually aroused[3]					
without vibration					
PE	4.4 ± 0.9	vib: F(1/19)=12.8,	5.9 ± 0.3	5.6 ± 0.3	dr: F(1/19)=3.2,
PE/ED	5.2 ± 0.7	p=0.002	5.3 ± 0.7	5.8 ± 0.5	p=0.087
Contr	4.6 ± 0.3		4.8 ± 0.2	4.9 ± 0.2,	vib: F(1/19)=18.6
with vibration					p<0.001
PE	6.4 ± 0.3		6.2 ± 0.4	6.5 ± 0.3	
PE/ED	6.0 ± 0.6		5.7 ± 0.6	6.2 ± 0.5	
Contr	5.6 ± 0.5		5.5 ± 0.4	6.2 ± 0.2	
How close to ejaculation[4]					
without vibration					
PE	1.8 ± 0.5	vib: F(1/19)=39.4,	1.9 ± 0.6	2.8 ± 0.7	gr: F(2/19)=2.6,
PE/ED	1.7 ± 0.5	p<0.001	3.0 ± 0.7	2.8 ± 0.9	p=0.0097
Contr	1.8 ± 0.4		1.6 ± 0.4	1.5 ± 0.3	dr: F(1/19)=3.7,
with vibration					p=0.07
PE	4.4 ± 0.7		2.8 ± 0.7	4.5 ± 0.8	vib: F(1/36)=9.6,
PE/ED	4.3 ± 1.0		4.8 ± 1.1	5.5 ± 0.7	p=0.032
Contr	4.2 ± 0.7		2.9 ± 0.7	3.1 ± 0.7	
Estimated penile response[5]					
without vibration					
PE	60 ± 17	vib: F(1/19)=11.6,	82 ± 9	76 ± 10	dr: F(1/19)=5.7,
PE/ED	50 ± 11	p=0.003	54 ± 14	70 ± 11	p=0.034
Contr	64 ± 9		62 ± 9	73 ± 5	vib: F(1/19)=4.7,
with vibration					p=0.044
PE	86 ± 6		79 ± 10	86 ± 7	
PE/ED	67 ± 11		65 ± 15	73 ± 12	
Contr	80 ± 5		70 ± 9	86 ± 5	
Strongest feeling in penis[6]					
without vibration					
PE	4.9 ± 1.0	vib: F(1/19)=20.0,	6.2 ± 0.4	5.8 ± 0.6	vib: F(1/19)=10.5,
PE/ED	4.2 ± 0.7	p<0.001	4.5 ± 0.8	5.3 ± 0.6	p=0.004
Contr	5.2 ± 0.4		5.1 ± 0.4	5.2 ± 0.3	grxdrxvib: F(3/19)=3.8,
with vibration					p=0.041
PE	6.8 ± 0.2		6.1 ± 0.4	6.9 ± 0.4	
PE/ED	6.2 ± 0.7		6.8 ± 0.7	6.5 ± 0.8	
Contr	6.2 ± 0.4		6.0 ± 0.5	6.5 ± 0.3	
Increase in penile circumference (mm)					
without vibration					
PE	14.0 ± 4.2	vib: F(1/19)=10.3,	19.1 ± 4.7	15.2 ± 3.1	vib: F(1/19)=8.7,
PE/ED	13.3 ± 5.5	p=0.005	17.0 ± 5.0	14.0 ± 3.9	p=0.008
Contr	12.9 ± 2.8		16.6 ± 3.0	21.1 ± 2.6	
with vibration					
PE	18.9 ± 3.6		19.2 ± 4.8	20.0 ± 4.1	
PE/ED	16.0 ± 5.3		19.7 ± 3.2	22.3 ± 4.8	
Contr	20.0 ± 3.0		22.4 ± 3.6	23.5 ± 2.3	

[1] 2-way analysis of variance, factors groups (gr) and type of stimulation (with or without vibration; vib). [2] 3-way analysis of variance, factors drugs (dr), gr and vib. [3] 1-not at all, 4-moderate, 7-very strong. [4] 1-not at all, 4-moderate, 7-very close, [5] 0-none, 100- full erection. [6] 1-none, 7-full erection

with primary premature ejaculation and controls. Marginal differences between sexually dysfunctional and control men were found in perceived quality of the

relationship (p=0.076) and current sex life (p=0.075) but no differences were noted in frequency of sexual intercourse.

The waking erectile assessment pretest data obtained in the laboratory are presented in table 9.2. Overall, vibration concomitant with visual erotic stimulation significantly increased the responses in all subjects. However, the groups did not differ in level of sexual arousal, closeness to ejaculation, estimated penile erection or measured increase in penile circumference. There was no significant interaction between groups and vibration. Although subjects with premature ejaculation and erectile dysfunction had the lowest erectile response from nocturnal penile tumescence measurements, this group did not differ significantly from men with primary premature ejaculation and controls. The latter 2 groups also did not differ.

Treatment phase

Drawing from the most relevant item on the daily logs, 2-way analysis of variance for estimated duration to reach orgasm or ejaculation with sexual activity (coitus or masturbation) revealed an effect of treatment ($F(1/16)=6.33$, $p=0.023$, mean plus or minus standard error 7.3 ± 1.5 minutes for clomipramine versus 4.5 ± 1.0 minutes for placebo), an effect of groups ($F(2/16)=5.09$, $p=0.019$) and a groups times treatment interaction ($F(2/16)=2.83$, $p=0.088$, premature ejaculation 5.2 ± 1.4 minutes, premature ejaculation plus erectile dysfunction 2.6 ± 1.0 minutes, and controls 10.0 ± 1.7 minutes). Post hoc analysis (Wilcoxon matched pairs signed ranks test) revealed a significant increase ($z=-2.11$, $p=0.035$) in duration to ejaculation from approximately 2 to 8 minutes in men with primary premature ejaculation (see figure 9.1), a statistically insignificant increase from approximately 9 to 11 minutes in controls and no change in men with premature ejaculation and erectile dysfunction.

Retrospective self-assessment at the end of each treatment phase revealed clomipramine effects on ejaculatory response similar to those revealed by daily log data. For example, regarding the item "orgasm sooner than wanted," further inspection of a significant (p=0.004) treatment times group interaction indicated that only men with primary premature ejaculation significantly increased control over orgasm or ejaculation with clomipramine. A similar trend, although not statistically significant, was observed in estimated in estimated duration of intercourse until orgasm or ejaculation or ejaculation in subjects with primary premature ejaculation during clomipramine treatment. There was no effect of

clomipramine on either measurement in men with premature ejaculation and erectile dysfunction and controls. There was also a significant improvement in judgement of the relationship with clomipramine therapy (5.8 ± 0.3) versus placebo (5.2 ± 0.3), with the largest increase occurring in men with primary premature ejaculation (5.4 to 6.6, table 9.1).

In contrast with items assessing ejaculatory response, items related to erectile function, or sexual desire or interest showed no variation among drug treatment phases (table 9.1). While several group differences in erectile items occurred, these were attributable mainly to the lower level of response reported by men with premature ejaculation and erectile dysfunction.

For waking erectile assessment measurements in treatment phase psychophysiological laboratory data were analyzed according to the variables of drug, groups and type of stimulation (table 9.2). There was a marginal drug effect on closeness to ejaculation ($p=0.07$). Subjects given placebo reported being closer to ejaculation during stimulation than when given clomipramine (3.3 ± 0.3 and 2.7 ± 0.3, respectively). There was a similar drug effect for self-reported sexual arousal ($p=0.087$), with lower values achieved with clomipramine than with placebo (5.6 ± 0.3 versus 5.9 ± 0.1, respectively). The strongest drug effect was found in estimated penile response ($p=0.034$). Mean estimated erection was lower with clomipramine (69.4 ± 4.3 compared to 77.8 ± 3.3 with placebo).

During waking erectile assessment, none of the 7 men with primary premature ejaculation experienced emission when given clomipramine, whereas 2 ejaculated during placebo treatment and 1 during the pretest period. Of 6 men with premature ejaculation and erectile dysfunction 3 ejaculated during both treatment phases (2 also during the pretest period). One of 6 controls ejaculated during all 3 visits.

Of lesser significance, group and stimulation (erotic video presented with or without penile vibration) effects occurred for several waking erectile assessment measurements (table 9.2). However, no interaction effects were detected.

During the clomipramine phase, significantly decreased nocturnal penile tumescence measurements were obtained. With the yellow collar Erectiometer penile circumference increased 22.4 ± 2.5 mm during the clomipramine phase and 27.6 ± 2.5 mm during the placebo phase, compared to 15.8 ± 1.9 and 23.8 ± 1.9, respectively, with the green Erectiometer noted. A significant ($p=0.051$) overall group difference was also noted with the yellow collar Erectiometer: 14.8

± 2.8, 26.3 ± 1.9 and 28.0 ± 2.6 for men with premature ejaculation and erectile dysfunction, primary premature ejaculation and controls, respectively.

Side effects

Generally, the drug was well tolerated without major side effects, and all patients complied with and completed the study. Reported side effects were dry mouth in 4 patients, fatigue or low energy in 8 and slight dizziness in 3. Of the controls 1 reported nausea, headache and excessive yawning, which disappeared after decreasing the dose to approximately half.

Drug discrimination

Of the 14 patients 9 correctly discriminated clomipramine from placebo treatment, 3 were incorrect in assumption and 2 did not know the difference. Of the 7 subjects in the sexually functional comparison group 3 correctly discriminated clomipramine from placebo, 1 was incorrect and 3 did not know the difference. The difference in correct identification of treatment phases between controls and dysfunctional men was not statistically significant.

DISCUSSION

In men with primary premature ejaculation, clomipramine effectively increased self-assessed ejaculatory latency. These men also reported greater control over ejaculation, as well as an improved sex life and relationship while on clomipramine therapy taken as needed. Such positive effects of clomipramine are consistent with several recent double-blind studies using comparable doses chronically in men with primary premature ejaculation (Girgis *et al.*, 1982, Segraves *et al.*, 1993, Althof *et al.*, 1995). Our study expands on previous investigations, not only by demonstrating the effectiveness of as needed administration of clomipramine but also by showing that its effects are specific to men with primary premature ejaculation. Neither sexually functional controls nor men with premature ejaculation and erectile dysfunction had comparable effects from identical clomipramine treatment. Furthermore, based on self-assessment, clomipramine did not postpone ejaculation indirectly through an effect of sexual desire.

When taken 12 to 24 hours before visiting the laboratory, clomipramine exhibited moderate effects on several sexual response measures during visual erotic stimulation. Consistent with daily log data, clomipramine decreased (marginally) the feeling of closeness to ejaculation, and this effect was most pronounced in men with primary premature ejaculation. In addition, we found lower subjectively assessed penile response (although not actual genital response), and attenuated sexual arousal to visual erotic and penile tactile stimulation with clomipramine therapy. Such findings are noteworthy because they suggest that clomipramine may exert its effect on ejaculation latency through multiple mechanisms. One mechanism may entail direct action on autonomic processes involved in the ejaculatory reflex. Another mechanism, suggested by our data, may involve diminished psychological arousal necessary for mediation of ejaculation. Indeed, this decreased subjective arousal along with weaker penile response (suggested by significantly lower nocturnal penile tumescence response in this study and a previous report (Steiger, 1988), as well as by nonsignificantly lower erectile response during waking erectile assessment) might partly explain why men with premature ejaculation and erectile dysfunction did not benefit from clomipramine. Pharmacological action, which has the potential for diminishing genital and/or subjective sexual arousal, and thereby exacerbating erectile problems already present (that is in the premature ejaculation plus erectile dysfunction group), may be inconsequential in its effect on ejaculatory response. For example, in men whose difficulty is predominantly with erection, premature ejaculation may represent a means of behavioral compensation, and in such instances pharmacotherapy may not well be suited to the disorder.

Because sex therapy using behavioral techniques and anxiety reduction has not always been successful in treatment of premature ejaculation, particularly in the long term (Althof, 1995b, Althof *et al.*, 1995), self-therapy with clomipramine has been suggested as an alternative for men with primary premature ejaculation and their partners (Zilbergeld, 1992). However, we agree with Rosen (1995) and Althof (1995a, 1995b) that pharmacotherapy should generally coincide with traditional sex therapy or some minimal counselling for the couple. Specifically, a strategy combining brief behavioral therapy with periodic treatment and/or retreatment with clomipramine may offer an effective option for men who wish to improve ejaculatory control. The fact that the drug need not be taken chronically but can be taken as needed, that is hours before sexual activity as in

our study and that of Riley and Riley (1993), is a particularly attractive feature of this approach to primary premature ejaculation therapy.

In conclusion, although satisfied with our results, we believe there is need for additional research given the potential limitation of our study due to small sample size, and the possibility of sampling error that might result.

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SUMMARY

We determined whether clomipramine taken as needed increases ejaculation latency in men with premature ejaculation and controls. The study included 8 patients with premature ejaculation, 6 with premature ejaculation and erectile dysfunction, and 8 controls. A prospective, double blind, placebo controlled, crossover design was used that included two 3-week periods with clomipramine and placebo. During treatment phases subjects took either 25 mg clomipramine or placebo as needed, that is 12 to 24 hours before anticipated sexual activity (coitus or masturbation). Subjects also visited the laboratory during these phases for evaluation of sexual response using visual erotic stimulation with and without vibration to the penis. Daily logs of sexual activities were maintained during treatment phases. Clomipramine significantly increased the latency to ejaculation during sexual activity (coitus or masturbation) from approximately 2 to 8 minutes in men with primary premature ejaculation. There were no significant effects in controls and men with premature ejaculation plus erectile dysfunction. Laboratory assessment indicated that men with primary premature ejaculation were better able to control ejaculatory response with clomipramine therapy. In these men clomipramine also resulted in increased satisfaction with sex life and relationship. Clomipramine inhibited nocturnal penile tumescence in all subjects. Clomipramine (25 mg as needed) effectively increases ejaculatory latency in men with primary premature ejaculation, while treatment is not effective in those with premature ejaculation and erectile dysfunction.

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Chapter 10

Fluoxetine and premature ejaculation: a double-blind, crossover, placebo controlled study*

SINCE THE EARLY 1970s, several case reports have described various sexual side-effects of the antidepressants clomipramine, fluoxetine and paroxetine (Balon, 1996). The most frequently reported sexual side-effect of these serotonergic reuptake inhibitors was delay or absence of orgasm, reported in both men and women (Balon, 1996). Eaton (1973) published the first open study with clomipramine to investigate a possible therapeutic effect of this orgasmic delay in men with premature ejaculation. Since then, several reports have confirmed the effects of various antidepressants on ejaculation latency in men with premature ejaculation (Balon, 1996, Althof, 1995, Althof *et al.*, 1995). However, only a few prospective, double-blind, crossover, placebo controlled studies have been published to date (Althof *et al.*, 1995, chapter 9). In one of those studies, we reported an increase in time to ejaculation and better control over ejaculation with clomipramine (25 mg, taken 12-24 hours before anticipated sexual activity) in men with premature ejaculation, but not in men with premature ejaculation and erectile dysfunction or in a comparison group of healthy control subjects (chapter 9). In the study presented here, with the use of a similar study design, we investigated the effects of the selective serotonin reuptake inhibitor fluoxetine on sexual function in men with premature ejaculation, in men with premature ejaculation and concomitant erectile dysfunction, in men with erectile dysfunction, and in control subjects. Fluoxetine was chronically taken in a relatively low dose (5-10 mg/day) to minimize the possible side effects.

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METHOD

Subjects

The experimental group consisted of 18 patients: 9 with premature ejaculation (PE) and 9 with premature ejaculation and erectile dysfunction (PE/ED). All met the DSM-IV (*Diagnostic and statistical manual of mental disorders*, 4th ed.) criteria for premature ejaculation. Erectile dysfunction was defined as the inability to achieve or maintain erection sufficient for sexual intercourse. There were two comparison groups: 7 patients with erectile dysfunction (ED), and 15 healthy, sexually functional volunteers (Contr). Patients were recruited from the urology outpatient clinic of a university hospital, and the sexually functional volunteers were recruited through word-of-mouth. All subjects fulfilled the following criteria: 18 years old or older, exclusively heterosexual orientation (to ensure homogeneity in the small groups and to maintain consistency by the use of only one set of erotic videos), sexual intercourse or masturbation at least once a week, consent of sexual partner when appropriate, and no history of somatic disease, previous surgery, drug use known to affect sexual function, or psychiatric problems, such as anxiety disorders or depression. The latter was determined in all subjects using a validated questionnaire (Zung, 1965). Further characterization of the groups is provided in table 10.1. Subjects gave their consent to participate after procedures and possible side effects were explained to them.

TABLE 10.1. Characteristics and pretreatment data (means ± SE) of men with primary premature ejaculation (PE), erectile dysfunction (ED), premature ejaculation secondary to erectile dysfunction (PE/ED) and healthy volunteers without sexual problems (Contr).

	PE	ED	PE/ED	Contr	p	LSD(5%)
Number of subjects	9	7	9	15[1]		
Mean age (years)	41.9 ± 4.6	54.6 ± 3.7	51.2 ± 2.7	41.3 ± 2.0	0.01	7.7
Age (range)	26-64	40-70	41-65	31-54		
Age of partner (years)	39.2 ± 2.7	51.7 ± 3.6	45.7 ± 3.3	39.1 ± 1.8	0.01	6.7
Duration of relationship (years)	11.9 ± 2.8	28.3 ± 3.6	19.9 ± 4.9	16.4 ± 2.2	0.02	8.2
Zung depression scale[2]	32.3 ± 1.9	32.3 ± 2.7	37.8 ± 2.7	28.1 ± 0.9	0.005	4.7
Getting an erection[3]	3.8 ± 0.3	2.0 ± 0.5	2.8 ± 0.2	4.5 ± 0.2	<0.001	0.7
Keeping an erection[3]	3.7 ± 0.4	1.7 ± 0.3	2.1 ± 0.3	4.3 ± 0.2	<0.001	0.7
Ejaculation latency with coitus (sec)	73 ± 22	360 ± 91	89 ± 22	535 ± 116	0.003	258

Data were subjected to one-way analysis of variance. P-values ≤ 0.10 are indicated. The Least Significant Difference (LSD(5%)) indicates the difference in means that is needed to reach a significant difference of $p \leq 0.05$. [1] one subject had no sexual relationship. [2] standard validated questionnaire (Zung, 1965). Score <40: no indication of depression. [3] 1-always a problem, 3-50% of the time, 5-never a problem

Before implementation of the prospective, randomized, double-blind, placebo controlled, crossover study, a pretest session was provided for initial evaluation an collection of data. This provided subjects the opportunity to adapt to the laboratory setting and procedures. Treatment phases consisted of three 4-week periods: placebo or fluoxetine treatment, washout, and crossover treatment. The dosages of fluoxetine were 5 mg/day for 2 weeks, followed by 10 mg/day for 2 weeks. During the washout period, no experimental treatment was given. Placebo and drug were assigned randomly.

After completion of the study, the unblinded code revealed that 13 patients began with fluoxetine and 12 patients with placebo. Of the control subjects, eight started with fluoxetine, and seven with placebo.

During the pretest session and at the end of each experimental 4-week phase (fluoxetine, washout and placebo), a series of self-assessments and objective measurements of sexual function were carried out. Self-assessment, based on an extensive structured interview, relied on an inventory of sexual function that consisted of 54 items regarding with quantitative and qualitative aspects of the sexual relationship and sexual activities, and incidence of sexual problems.

Objective assessment of genital response was achieved with nocturnal penile tumescence (NPT) and waking erectile assessment procedures (Janssen *et al.*, 1994, Rowland *et al.*, 1994, Rowland and Slob, 1995, Slob *et al.*, 1990, chapter 8). In the laboratory (figure 9.2), waking erectile assessment with visual erotic stimulation and penile tactile stimulation was conducted using penile measurements with an Erectiometer (figure 9.1) with attached minivibrator around the tip of the penis was used to measure increase in penile circumference), and subjective assessment of arousal and genital response (Rowland and Slob, 1995). The Erectiometer was read by the experimenter. The lap of the subject was covered to prevent visual genital feedback. Two different videos, each appr. 8 min long were used in each session, and different sets of videos were used in the different sessions (pretest and three subsequent lab visits). The videos were similar in types of explicit heterosexual activities portrayed.

Each subject was first exposed to an erotic video without concomitant vibration and then exposed to a second erotic video with concomitant vibration to the underside of the tip of the penis. This order of presentation was selected because previous research indicated greater arousal and genital response to the combination of the video and penile vibration (Rowland *et al.*, 1994, chapter 9).

TABLE 10.2. Questionnaire and nocturnal penile tumescence data (means ± SE) before and during fluoxetine and placebo treatment. PE: primary premature ejaculation, ED: erectile dysfunction, PE/ED: premature ejaculation secondary to ED, Contr: healthy volunteers without sexual dysfunction.

Item and description	No. Pts.	Pretest mean ± SE	Statistics[1] F, p-value	No. Pts.	placebo mean ± SE	fluoxetine mean ± SE	Statistics[2] F, p-value
Relationship score[3]							
PE	9	4.9 ± 0.6		9	4.5 ± 0.6	5.6 ± 0.4	dr: F(1/36)=2.9,
ED	7	6.0 ± 0.3	F(3/35)=3.2,	7	6.2 ± 0.2	6.2 ± 0.2	p=0.099
PE/ED	9	5.7 ± 0.2	p=0.035	9	5.6 ± 0.3	5.4 ± 0.3	gr: F(3/36)=2.6,
Contr	14	6.2 ± 0.1		15	5.7 ± 0.3	6.0 ± 0.2	p=0.07
Current sex life score[4]							
PE	9	3.2 ± 0.5		9	3.4 ± 0.5	3.6 ± 0.4	
ED	7	4.9 ± 0.6	F(3/36)=8.0,	7	5.2 ± 0.3	4.6 ± 0.3	
PE/ED	9	3.1 ± 0.3	p<0.001	9	3.3 ± 0.3	3.3 ± 0.3	gr: (3/36)=10.5,
Contr	15	5.1 ± 0.3		15	4.9 ± 0.3	5.1 ± 0.2	p<0.001
Frequencies/week							
Morning erections							
PE	9	3.1 ± 0.8		9	3.1 ± 0.9	3.3 ± 0.6	
ED	7	2.1 ± 0.9		7	1.7 ± 1.0	2.2 ± 0.9	
PE/ED	9	2.4 ± 0.6		9	1.4 ± 0.3	3.1 ± 0.6	
Contr	15	3.3 ± 0.6		15	3.5 ± 0.6	3.3 ± 0.6	
Intercourse							
PE	9	1.5 ± 0.5		9	1.3 ± 0.8	1.2 ± 0.4	
ED	7	1.0 ± 0.3		7	1.1 ± 0.4	0.9 ± 0.4	
PE/ED	9	1.4 ± 0.4		9	0.9 ± 0.2	1.4 ± 0.4	
Contr	15	1.6 ± 0.2		15	1.6 ± 0.3	1.7 ± 0.3	
Nocturnal penile tumescence[5] (mm)							
PE				8	24.1 ± 4.8	22.2 ± 3.8	
ED	(not measured)			6	17.5 ± 4.2	22.0 ± 3.0	
PE/ED				9	13.2 ± 2.1	14.3 ± 1.2	gr: F(3/34)= 5.1,
Contr				15	25.7 ± 2.2	27.6 ± 2.4	p= 0.005
Sexual difficulties score							
Getting an erection when wanted[6]							
PE	9	3.8 ± 0.3		9	4.1 ± 0.4	3.9 ± 0.4	
ED	7	2.0 ± 0.5	F(3/36)= 13.7,	7	2.0 ± 0.4	1.9 ± 0.4	
PE/ED	9	2.8 ± 0.2	p<0.001	9	2.8 ± 0.4	3.2 ± 0.5	gr:F(3/36)= 16.4
Contr	15	4.5 ± 0.2		15	4.6 ± 0.1	4.7 ± 0.1	p<0.001
Keeping an erection when wanted[6] -During foreplay							
PE	9	3.7 ± 0.4		9	3.7 ± 0.4	3.4 ± 0.5	gr: F(3/36)= 2.0,
ED	7	1.7 ± 0.3	F(3/36)=17.9,	7	3.0 ± 0.6	2.1 ± 0.4	p<0.001
PE/ED	9	2.1 ± 0.4	p<0.001	9	1.7 ± 0.3	2.0 ± 0.3	
Contr	15	4.3 ± 0.2		15	4.5 ± 1.3	4.3 ± 0.2	dr x gr: p=0.091
Keeping an erection when wanted[6] -During intercourse							
PE	8	3.9 ± 0.4		6	3.3 ± 0.8	3.2 ± 0.6	
ED	6	2.2 ± 0.5	F(3/33)=17.4,	5	3.4 ± 0.7	3.2 ± 0.2	
PE/ED	9	2.1 ± 0.4	p<0.001	8	1.8 ± 0.3	2.5 ± 0.5	gr:F(3/30)= 13.9
Contr	15	4.6 ± 0.1		15	4.8 ± 0.1	4.6 ± 0.1	p<0.001
Fear of Failure[7]							
PE	9	2.8 ± 0.6		9	3.1 ± 0.6	2.7 ± 0.3	
ED	7	3.6 ± 0.6	F(3/36)=5.3,	7	3.7 ± 0.6	3.7 ± 0.4	
PE/ED	9	2.4 ± 0.5	p=0.004	9	2.8 ± 0.5	2.9 ± 0.6	gr: F(3/35)= 5.5,
Contr	15	4.5 ± 0.2		14	4.5 ± 0.2	4.5 ± 0.2	p= 0.003

[1] 1-way analysis of variance. [2] 2-way analysis of variance, factors drugs (dr) and groups (gr). [3] 1-very unhappy, 4-neutral, 7-very happy. [4] 1-very bad, 4-neutral, 7-excellent. [5] mean increase in circumference for 2 nights. [6] 1-always a problem, 3-50% of the time, 5-never a problem. [7] 1-always, 3-50% of the time, 5-never

Thus, the likelihood of ejaculation during the initial stimulus session (video only) was minimized. After each video, the subjects indicated whether they had ejaculated, and they completed a number of self-report items assessing sexual, genital, and ejaculatory response during the stimulus period.

In addition to the above laboratory assessments, subjects also completed short daily logs about various sexual activities, with particular attention paid to ejaculatory response. The time to ejaculation with coitus was defined as the estimated time from vaginal intromission to ejaculation; with masturbation it was defined as estimated time from start of masturbation to ejaculation. Patients and control subjects were asked to attempt sexual activity (coitus or solitary masturbation) at least once a week. If a subject did not have intercourse at least once in every treatment phase, the data on masturbation were used for analysis of ejaculation latency data.

Statistical Analysis

During the two treatment phases, a two-way analysis of variance (ANOVA) for a mixed factorial design with one between factor (group) and one repeated measure (placebo vs. fluoxetine) was used to determine effects on measurements of sexual function drawn from three sources (daily logs, retrospective self-assessment at the end of each treatment phase and NPT). Because waking erectile assessment sessions included a third variable, type of stimulation, a three-way ANOVA was used.

Data obtained from daily logs in the treatment phase (2 weeks of 5 mg/day fluoxetine followed by 2 weeks 10 mg/day fluoxetine) did not indicate any difference between 5 mg/day fluoxetine versus 10 mg/day fluoxetine. Therefore, these data were combined for further analysis.

With respect to the time to ejaculation with coitus or masturbation, data were compared using the paired t-test, after ensuring that no significant carryover or period effects occurred (Hills and Armitage, 1979). In this analysis data were logarithmically transformed to obtain an approximate normal distribution.

In general, two tailed p-values of ≤ 0.10 are indicated, with p-values of ≤ 0.05 considered to be statistically significant. If there was a statistically significant effect, the least significant difference (LSD) test was used to make pairwise comparisons between means (Kirk, 1968).

TABLE 10.3. Psychophysiological data (means ± SE) obtained in the laboratory during visual erotic stimulation without and with concomitant vibrotactile stimulation to the penis. PE: primary premature ejaculation, ED: erectile dysfunction, PE/ED: premature ejaculation secondary to ED, Contr: healthy comparison group.

Item and description	Pretest mean ± SE	Statistics[1] F, p-values	placebo mean ± SE	fluoxetine mean ± SE	Statistics[2] F, p-value
How sexually aroused[3]					
without vibration					
PE	4.0 ± 0.6	gr: F(3/36)=3.0,	4.8 ± 0.6	4.4 ± 0.5	gr: F(3/36)=3.2,
ED	3.6 ± 0.6	p=0.042	4.0 ± 0.6	3.7 ± 0.6	p=0.034
PE/ED	4.6 ± 0.5		4.7 ± 0.5	4.6 ± 0.4	
Contr	4.9 ± 0.2	vib: F(1/36)= 3.9,	5.5 ± 0.2	5.5 ± 0.2	vib: F(1/36)=21.1,
with vibration		p=0.055			p<0.001
PE	4.7 ± 0.6		5.4 ± 0.4	5.0 ± 0.3	
ED	4.0 ± 0.6		5.0 ± 0.3	4.3 ± 0.7	
PE/ED	4.8 ± 0.5		6.0 ± 0.3	5.3 ± 0.6	
Contr	5.6 ± 0.2		5.7 ± 0.3	5.8 ± 0.2	
Control over ejaculation[3]					
without vibration					
PE	5.9 ± 0.6		6.1 ± 0.5	5.2 ± 0.7	gr: F(3/36)=8.3,
ED	5.3 ± 0.8		5.4 ± 0.9	5.3 ± 0.8	p<0.001
PE/ED	4.8 ± 0.7		4.4 ± 0.7	4.3 ± 0.7	
Contr	6.5 ± 0.3		6.6 ± 0.2	6.7 ± 0.2	vib: F(1/36)=9.6,
with vibration					p=0.004
PE	4.3 ± 0.9		3.7 ± 0.8	2.4 ± 0.7	
ED	5.9 ± 0.9		6.1 ± 0.5	5.7 ± 0.7	gr x vib: F(3/36)=3.3,
PE/ED	4.8 ± 0.7		2.6 ± 0.6	4.2 ± 0.8	p=0.032
Contr	6.1 ± 0.4		5.7 ± 0.5	6.0 ± 0.4	
Estimated penile response[4]					
without vibration					
PE	47 ± 11	gr: F(3/36)=4.4,	45 ± 12	52 ± 11	gr: F(3/36)=7.3,
ED	25 ± 10	p=0.01	41 ± 11	31 ± 10	p=0.001
PE/ED	38 ± 10		32 ± 8	36 ± 12	
Contr	64 ± 6	vib: F(1/36)=7.4,	76 ± 5	72 ± 6	vib: F(1/36)=16.2,
with vibration		p=0.01			p<0.001
PE	56 ± 14		58 ± 11	75 ± 10	
ED	32 ± 8		40 ± 9	36 ± 12	gr x dr: p=0.058
PE/ED	49 ± 8		55 ± 8	47 ± 11	
Contr	74 ± 6		84 ± 3	82 ± 4	
Strongest feeling in penis[5]					
without vibration					
PE	3.8 ± 0.5	gr: F(3/36)=6.0,	3.6 ± 0.7	4.0 ± 0.8	gr: F(3/36)=5.9,
ED	2.1 ± 0.5	p=0.002	3.1 ± 0.9	2.7 ± 0.7	p=0.002
PE/ED	3.4 ± 0.7		2.9 ± 0.5	3.1 ± 0.7	
Contr	5.0 ± 0.4	vib: F(1/36)=4.3,	5.4 ± 0.3	5.3 ± 0.4	vib: F(1/36)=20.6,
with vibration		p=0.046			p<0.001
PE	4.3 ± 0.8		4.8 ± 0.7	5.1 ± 0.6	
ED	2.7 ± 0.5		3.6 ± 0.6	3.3 ± 0.8	
PE/ED	3.7 ± 0.6		4.6 ± 0.6	4.0 ± 0.7	
Contr	5.6 ± 0.4		6.1 ± 0.2	5.9 ± 0.2	
Increase in penile circumference (mm)					
without vibration					
PE	12.6 ± 3.3	gr: F(3/36)=6.1,	15.1 ± 4.6	18.8 ± 4.4	gr: F(3/36)=7.1,
ED	6.5 ± 2.5	p=0.002	6.1 ± 2.4	9.6 ± 2.7	p=0.001
PE/ED	13.9 ± 5.6		8.7 ± 2.8	16.7 ± 3.4	
Contr	23.7 ± 2.7	gr x vib: F(3/36)=2.9,	23.7 ± 2.8	26.8 ± 2.2	dr: F(1/36)=15.2,
with vibration		p=0.048			p<0.001
PE	17.4 ± 4.2		14.6 ± 5.4	22.1 ± 4.8	
ED	3.7 ± 1.4		7.1 ± 2.8	8.7 ± 2.6	
PE/ED	12.4 ± 4.7		10.9 ± 5.0	15.9 ± 4.5	
Contr	26.5 ± 2.5		26.1 ± 2.8	30.3 ± 2.8	

[1] 2-way analysis of variance, factors groups (gr) and type of stimulation (with or without vibration; vib). [2] 3-way analysis of variance, factors drugs (dr), gr and vib. [3] 1-not at all, 4-moderate, 7-very strong. [4] 0-none, 100- full erection. [5] 1-none, 7-full erection

RESULTS

Pretreatment data

Table 10.1 indicates demographic details and characteristics of sexual function in the four groups of men. Self-estimated latency to ejaculation was significantly shorter in the PE and PE/ED groups, compared to the ED group and the control subjects ($p=0.003$). Men with ED and PE/ED reported to have more difficulties in getting ($p<0.001$) and keeping ($p<0.001$) an erection when desired. Table 10.2 shows that patients with PE reported having a less satisfactory relationship than the control subjects (LSD(1%)= 1.0), patients with PE and PE/ED reported to have a lower appreciation of their current sex life than ED and controls (LSD(5%)=0.9), and PE and PE/ED groups had more fear of failure than the healthy group (LSD(5%)=1.1), and the PE/ED had also more fear of failure than more than the ED group.

Treatment phase

Time to ejaculation with sexual activity (coitus or masturbation) in the different groups is shown in figure 10.1. One subject of the PE group did not have sexual intercourse during treatment phases, therefore his masturbation activity which was relatively high was used (placebo: 615 seconds, fluoxetine: 855 seconds). In the PE/ED group a significant increase in ejaculation latency ($p=0.03$) occurred with fluoxetine treatment. In the PE group, the mean time to ejaculation with fluoxetine compared with placebo was nearly doubled (190%; 95% probability interval: 80-450%), but a statistically significant difference was not reached ($p=0.13$). There was no statistically significant difference between the PE and the PE/ED groups in an increase in time to ejaculation. When combining the PE and PE/ED groups, fluoxetine significantly increased the mean time to ejaculation with sexual activity ($p=0.007$).

The above results were analyzed further to determine whether the proportion of subjects within each group showing an increase in ejaculation latency was significant. Of the PE group, six of seven patients reported an increase in the time to ejaculation with fluoxetine and one a decrease (McNemar's Test: n.s.). Of the men in the PE/ED group, six of eight patients reported an increase, one a decrease and one patient no change with fluoxetine. Four of six patients in the ED group reported a decrease in the time to ejaculation, one an increase, and one an inability to reach ejaculation during fluoxetine treatment (masturbation, two

attempts). In the control group, seven of fifteen reported an increase and eight a decrease in the time to ejaculation with fluoxetine (figure 10.1).

Retrospective self-assessment at the end of each treatment phase revealed no statistically significant drug effect (table 10.2). For judgement of the relationship, of current sex life, and items related to erectile function, the differences

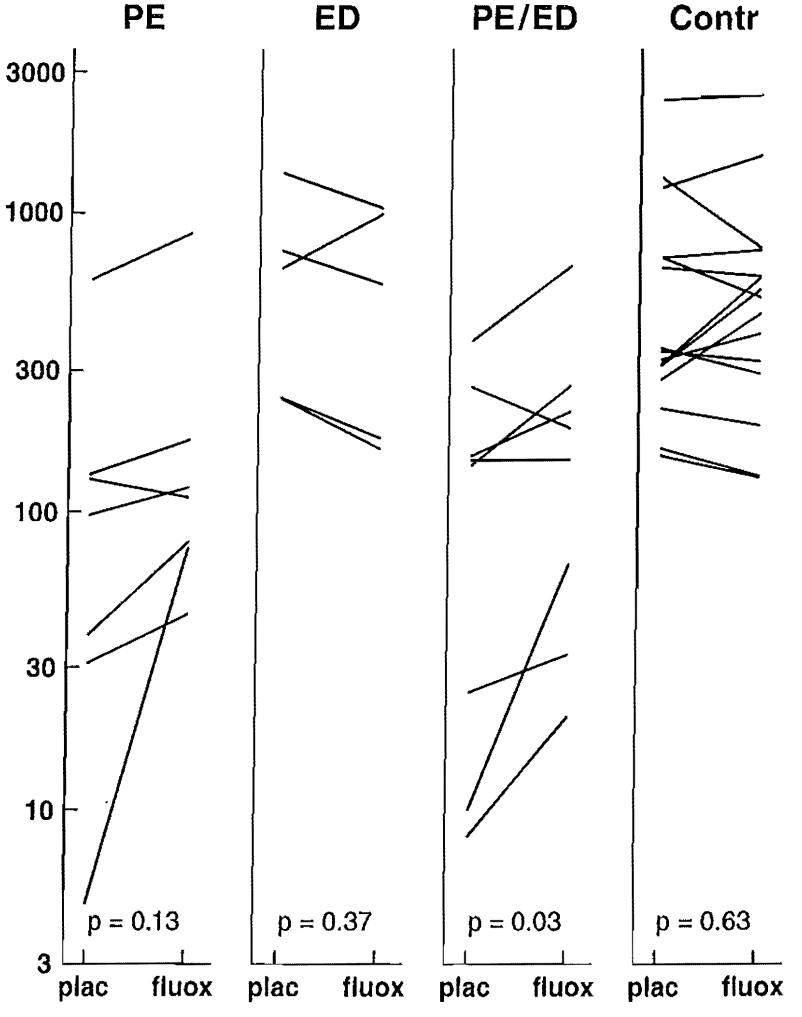

FIGURE 10.1. Self-estimated duration to ejaculation with sexual activity (coitus or masturbation). In the double-blind, crossover design, subjects received placebo (plac) and 5-10 mg/day fluoxetine (fluox) treatment. PE: 7 men with premature ejaculation, ED: 5 men with erectile dysfunction, PE/ED: 8 men with premature ejaculation secondary to erectile dysfunction, and Contr: 15 volunteers without sexual dysfunction. One subject of the PE group did not have sexual intercourse during treatment phases, therefore his masturbation data were used which were relatively high (plac 615 sec, fluox: 855 sec).

between the groups were similar to the pretreatment data, but no significant effects of fluoxetine treatment were found.

For waking erectile assessment measurements the treatment phase psychophysiologic laboratory data were analyzed according to the variables of drug, groups and type of stimulation (table 10.3). The only significant effect of fluoxetine was found in increase in penile circumference in all groups ($p<0.001$; measured with the Erectiometer). Consistent with previous data obtained from our laboratory, group and stimulation (erotic video without or with penile vibration) effects occurred for all subjective waking erectile assessment parameters as can be seen in table 10.3.

During waking erectile assessment in the laboratory, five of nine men with PE experienced ejaculation during fluoxetine treatment, whereas four ejaculated during placebo treatment and three during the pretest period. Of nine men with PE/ED, two ejaculated during both treatment phases, and three during the pretest period. One of the seven men with ED ejaculated during placebo treatment. Of fifteen men of the control group, one ejaculated during all three visits, and one ejaculated during placebo treatment.

Measurement of NPT revealed an effect of groups ($p=0.005$, table 10.2), but no effect of fluoxetine. Post-hoc analysis revealed that men with PE/ED (13.8 mm) differed from both PE (23.2 mm) and control groups (26.6 mm; LSD(5%)= 9.2). ED (19.8 mm) and control subjects did not differ significantly.

Side effects and drug discrimination

Generally, fluoxetine was well tolerated without major side effects, and all subjects completed the study without alterations in the drug doses. Patients reported side effects with fluoxetine as follows: dry mouth (three), increased libido (two); loose stools (two); slight palpitations (one); episodes of pain in penis (one); and dizziness (one). Of the healthy control subjects, two complained of altered sleeping pattern and one of sweating. With placebo, the reported side effects were increased libido (one), decreased libido (one), burning sensation with micturition (one) and change in stools (one).

Of the 25 patients seven correctly discriminated fluoxetine from placebo treatment, seven erred and eleven did not know. Of the healthy comparison group ($n=15$), nine correctly discriminated fluoxetine from placebo, two erred and four did not know. There was no difference between the groups in drug discrimination.

DISCUSSION

In men with premature ejaculation and erectile dysfunction (PE/ED), fluoxetine effectively increased self-assessed ejaculatory latency. In men with premature ejaculation (PE), the time to ejaculation with sexual activity increased in all but one individual, but this change did not reach statistical significance. Retrospective self-assessment of various items at the end of each treatment phase did not reveal any additional beneficial effects of fluoxetine on (quality of) sexual function and in particular, ejaculatory response, in any of the groups.

Several case reports have been published on the effects of fluoxetine on orgasmic delay in up to 16% of both men and women (Herman *et al.*, 1990, Musher, 1990, Power-Smith, 1994). In these reports, all patients were treated for depression or other psychiatric disorders, and, therefore, these groups were not comparable to the dysfunctional or healthy men examined in our study. In a double-blind study, Kara and co-workers (1996) found a beneficial effect of fluoxetine (40 mg/day) in men with PE. In these patients, the ejaculation latency time increased "noticeably" after 4 weeks of treatment from 25 to 180 seconds with fluoxetine. Two of the 17 patients were excluded from this study because of excessive nausea and insomnia. Although we used much lower dosages of fluoxetine (5 and 10 mg/day) in our crossover study, and therefore subjects suffered from fewer side effects, our results on ejaculatory latency are comparable. In a recently published open clinical trial with fluoxetine, Lee *et al.* (1996) found an increase in self-assessed median time to ejaculation from 0.91 minutes (baseline) to 9.64 minutes (20-60 mg/day fluoxetine).

A new finding in our study is that during erectile assessment in the lab, fluoxetine stimulated erectile response in all groups, as measured objectively with the Erectiometer. However, this was apparently not noted by the subjects, because items relevant to erection on the questionnaire (self-estimated percentage of full erection) did not reveal such an effect of fluoxetine. Murray and Hooberman (1993) reported a case of prolonged erections with fluoxetine, and another case was published on the occurrence of prolonged erections with fluoxetine in the absence of sexual stimulation (Swenson, 1993). Further research is required to study the effects of fluoxetine on erections in non-depressed men.

In conclusion, fluoxetine (5-10 mg/day) seems to be a safe drug that increases the time to ejaculation in patients with premature ejaculation. Future investigations with fluoxetine might consider larger sample sizes, an "as needed"

design (i.e., a few hours before anticipated sexual activity), and a higher dose (e.g., 20 mg). In the treatment of premature ejaculation, the option of pharmacological intervention is becoming increasingly popular. However, we emphasize that drug treatment should be combined with sexual counselling of the patient and the partner to maximize its beneficial effects. Furthermore, careful follow-up should be undertaken because data on the long-term effects of serotonin reuptake inhibitors on premature ejaculation are not yet available.

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SUMMARY

The purpose of this study was to investigate the effect of fluoxetine on sexual function in males with premature ejaculation and/or erectile dysfunction and controls, we conducted a prospective, double-blind, placebo controlled, crossover study. There were 4 groups: 1. premature ejaculation (PE, n=9), 2. PE and erectile dysfunction (PE/ED, n=9), 3. erectile dysfunction (n=7) and 4. healthy, sexually functional controls (n=15). The study consisted of three 4-week periods: fluoxetine, wash-out, placebo (or *vice versa*). Fluoxetine began at 5 mg/day for 2 weeks, followed by 10 mg/day for 2 weeks. At weeks 0, 4, 8 and 12, subjects visited the lab for evaluation of sexual function and assessment of erectile response, ejaculation and sexual arousal to visual erotic stimulation without and with concomitant vibrotactile stimulation to the penis. At home, daily logs for sexual activities and feelings of well-being were maintained, and nocturnal penile tumescence was measured. The latency to ejaculation increased significantly in the PE/ED group ($p=0.03$) and in the PE and the PE/ED group taken together ($p=0.007$), but not in the PE group alone. Fluoxetine stimulated objectively but not subjectively measured erectile response during laboratory

assessment in all groups. No major side-effects were reported. In conclusion, fluoxetine (5-10 mg/day) was effective in increasing latency to ejaculation in patients with premature ejaculation (PE and PE/ED combined).

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Chapter 11

Summary, general discussion and conclusions

THE OBJECTIVE OF THIS THESIS is to investigate the effects of the neurotransmitter serotonin (5-HT) and serotonergic agents on the ejaculation behavior in male rats and human males. In *chapter 1* a definition of ejaculation is given, the aim of this thesis is presented and a general outline of the contents of the different chapters is given.

In *chapter* 2, some basics about neurotransmitters are explained. A neurotransmitter, like serotonin, is a chemical substance that communicates between nerve cells. When an adequate impulse (action potential) reaches the terminal nerve ending, a chemical substance, the neurotransmitter is released from the vesicles into the synaptic cleft, that can react with a special molecule on the next nerve cell, the receptor. As a result of this reaction, a new action potential is created on the second nerve cell. There are also receptors that inhibit the formation of a new action potential, like 5-HT_{1A} receptors. After transfer of the action potential to the next nerve cell, the neurotransmitter is disintegrated, or actively transported back into the neurotransmitter vesicles in the terminal nerve endings for reuse (reuptake). By using agents that imitate neurotransmitters (agonists), effects of certain receptors can be investigated. When using agents that block certain receptors (antagonists), also information about the function of this receptor can be investigated. In the animal experiments described in this thesis, two 5-HT_{1A} receptor agonists are used: 8-OH-DPAT and flesinoxan. These agents decrease serotonin levels in certain parts of the brain. In the human studies, two serotonin reuptake inhibitors are used: clomipramine and fluoxetine, which increase the serotonin levels in the synaptic cleft.

In the remaining part of chapter 2, a historical review is presented of the classification of serotonin receptors and details are given about the agents that are used in the different experiments.

PART 1

In *chapter 3* the sexual behavior of the normal male rat is described. It consists of mounts, intromissions and ejaculations. An adult, sexually experienced male rat when behaviorally tested with an estrous female will ejaculate after 6 or 7 intromissions. This is followed by a period of sexual inactivity (post-ejaculatory interval).

The sexual behavior of the male rat changes dramatically after administration of 8-OH-DPAT. Not only the latency to ejaculation decreases from about 5 minutes to a few seconds, but the copulation pattern is also altered: prior to first ejaculation, no or only a few mounts or intromissions are observed. Therefore, this behavior is sometimes called "premature ejaculation," in analogy to the well known sexual dysfunction in the human male. In this chapter, the effects of other agents on sexual behavior in the male rat are also reviewed, like serotonin, flesinoxan and dopamine.

In the remaining chapters of Part 1 experiments are described that were performed in our laboratory to investigate the effects of 5-HT_{1A} receptor agonists on male rat sexual behavior. In *chapter 4*, four experiments are presented. In the first experiment active and inactive middle-aged male rats (12-14 months old) are compared. Administration of 8-OH-DPAT stimulates sexual behavior in these rats, and the difference in ejaculation frequencies disappears after treatment with 8-OH-DPAT.

In the next experiment, the ejaculation is observed in adult male rats treated with 8-OH-DPAT. With a dosis of 0.4 mg/kg, 50-55% of the rats ejaculate with the first or second intromission ("premature ejaculation"), and 25-35% of the animals ejaculate extravaginally. Microscopical inspection of the seminal plug does not show any gross abnormalities of the semen.

In the third experiment, an estrous female is tested in a three compartment box, with a saline treated tethered male rat in one and a 8-OH-DPAT treated tethered male in another lateral compartment. 8-OH-DPAT does not make males more attractive for the estrous female, as judged by time spent in their vicinity. However, estrous females receive significantly more ejaculations from the tethered 8-OH-DPAT males than from the control males.

When a female rat is put in a test cage with an estrous female conspecific, she can display mounting behavior. In the final experiment described in chapter 4, 8-

OH-DPAT stimulates mounting behavior in the female rat only when they are long-term treated with testosterone.

To investigate if it is necessary to have physiological serum levels of testosterone for 8-OH-DPAT to exert its stimulating properties on male rat sexual behavior, an experiment is performed that is presented in *chapter 5*. Twelve adult, sexually experienced male rats are tested with an estrous female rat before and up to 1 year after castration. Without adequate testosterone levels, it is not possible to stimulate sexual behavior with 8-OH-DPAT. Twenty weeks after castration they are chronically treated with a subcutaneously implanted testosterone capsule, to reach physiological serum levels of testosterone. The ability of 8-OH-DPAT to stimulate sexual behavior is now restored. Therefore it is concluded that testosterone is required for the activating effects of 8-OH-DPAT on sexual behavior in male rats.

In the final chapter of Part 1, *chapter 6*, two 5-HT_{1A} receptor agonists are compared in their effects on male rat sexual behavior. Two groups of rats are used: normal rats and rats, neonatally treated with the aromatase inhibitor ATD, that results in inhibited sexual behavior on adult age. With flesinoxan, also a selective 5-HT_{1A} receptor agonist, the ejaculation frequency increases and also the number of animals displaying this behavior. However, no "premature ejaculations" are observed (ejaculation with the first or second intromission). In a second experiment, flesinoxan is compared with 8-OH-DPAT. Again, no premature ejaculations are observed after administration of flesinoxan, while animals in both groups (ATD and controls) do so after treatment with 8-OH-DPAT. Because flesinoxan stimulates frequencies, but does not alter the pattern of sexual behavior in male rats, it could be called a prosexual drug. In contrast, with an adequate dose of 8-OH-DPAT, male rats become premature ejaculators.

PART 2

In the second part of this thesis the human studies are described. In *chapter 7* a review is given about premature ejaculation and pharmacological treatment with serotonergic antidepressants. The first reports on sexual side effects of serotonin reuptake inhibiting (tricyclic) antidepressants, like clomipramine, date from the seventies. One of the reported side effects was the unability to reach orgasm. Since these reports, several studies have been performed to investigate the effects

of these antidepressant in patients with premature ejaculation. Most reports show beneficial effects of such drugs. However, double-blind, crossover, placebo controlled studies in patients and healthy controls are lacking. Moreover, only few objective laboratory data are available on the sexual function of men with premature ejaculation and/or erectile dysfunction and controls, when treated with serotonin reuptake inhibitors.

On the assumption that penile sensitivity might be greater in men with premature ejaculation, the study presented in *chapter 8* investigates penile threshold (sensitivity) to vibrotactile stimulation in men with premature ejaculation, with erectile dysfunction, with a combination of the two, or in healthy controls. Men with premature ejaculation show thresholds similar to that of controls, while men with erectile dysfunction, or combined erectile dysfunction and premature ejaculation, show elevated thresholds. Thus, men with premature ejaculation do not have penile hypersensitivity, but men with erectile dysfunction, with or without premature ejaculation, have a decreased sensitivity.

The effect of clomipramine (Anafranil®) on sexual function in men with premature ejaculation and controls is described in *Chapter 9*. Eight men with premature ejaculation, 6 with premature ejaculation and erectile dysfunction and 8 healthy controls took in a 3 week period clomipramine (25 mg), 12-24 hours before anticipated sexual activity, followed by a 3 week period in which placebo was taken on demand (or *vice versa*). At the start of the prospective, double-blind, placebo controlled crossover study and at the end of each period the subjects visit the laboratory. In the laboratory sexual function is assessed by measuring erectile response using visual erotic stimulation without or with vibrotactile stimulation to the penis. At home, daily logs of sexual activities and feelings of well-being are maintained during treatment phases. Nocturnal penile tumescence is measured during 2 nights using an Erectiometer®. With clomipramine, time to ejaculation with coitus or masturbation increases in men with premature ejaculation, but not in men with premature ejaculation plus erectile dysfunction or in controls. Laboratory assessment indicated that men with premature ejaculation were better able to control ejaculatory response with clomipramine. In these men clomipramine also resulted in an increased satisfaction with sex life and relationship. Clomipramine inhibited nocturnal penile tumescence in all subjects.

In *chapter 10* the final experiment of this thesis is described. In a double-blind, placebo controlled crossover study the effects are investigated of the selective serotonin reuptake inhibitor fluoxetine (Prozac®) on sexual function in 9 men with premature ejaculation, 9 with premature ejaculation plus erectile dysfunction, 7 with erectile dysfunction alone and in 15 healthy volunteers without sexual problems. There are 3 periods of 4 weeks: fluoxetine (5-10 mg/day), no treatment and placebo (or *vice versa*). Assessment of sexual function at home and in the lab is very similar to the clomipramine study (see chapter 9). Latency to ejaculation increases in men with premature ejaculation plus erectile dysfunction and in the combined group of men with premature ejaculation without and with erectile dysfunction, but not statistically significant in the group of men with premature ejaculation alone. Fluoxetine stimulates objectively but not subjectively erectile response during laboratory assessment in all groups. No major side-effects were reported, and nocturnal penile erections did not change during fluoxetine treatment.

GENERAL DISCUSSION

In Part 1 of this thesis the influence of serotonergic agents on male rat sexual behavior is described. Administration of 8-OH-DPAT, a 5-HT_{1A} receptor agonist that lowers 5-HT levels in some parts of the rat brain, causes male rats to ejaculate with the first or second intromission. Maybe, this could be used as an animal model for the human sexual dysfunction premature ejaculation.

Behavioral experiments in rats with selective 5-HT reuptake inhibitors have shown inhibition of male rat sexual behavior (see chapter 3). The administration of serotonin reuptake inhibitors results in a higher concentration of 5-HT in the synapse with a concomitant decrease of various ejaculatory behaviors. The administration of 5-HT_{1A} receptor agonists results in a lower concentration of 5-HT in the synapse with a subsequent increase in ejaculatory behaviors. Apparently, the 5-HT levels in the synapse determine the ejaculatory latency. It remains to be demonstrated whether or not the effects of 8-OH-DPAT and flesinoxan on sexual behavior in male rats could be antagonized by simultaneous administration of a selective serotonin reuptake inhibitor.

It is unlikely that serotonin is the only neurotransmitter responsible for control of ejaculatory behavior. In the literature, there are several studies indicating the

significance of other neurotransmitters on ejaculatory behavior. Comparing the different studies, no unequivocal model can be made for the neuronal control of ejaculatory behavior in the rat.

Another 5-HT_{1A} receptor agonist, flesinoxan, stimulates male rat sexual behavior without altering the copulatory pattern. Therefore, flesinoxan could be called a prosexual drug for male rats. The difference in facilitation of male rat sexual behavior between the two selective 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT is depicted in figure 11.1. An explanation for this phenomenon may be an administration route effect or a different pharmacological affinity pattern. The latter possibility would imply a role for dopamine, since flesinoxan has a limited co-affinity for the D_2 receptor (Olivier *et al.*, 1995). Administration of various dopamine receptor agonists facilitate several aspects of copulatory

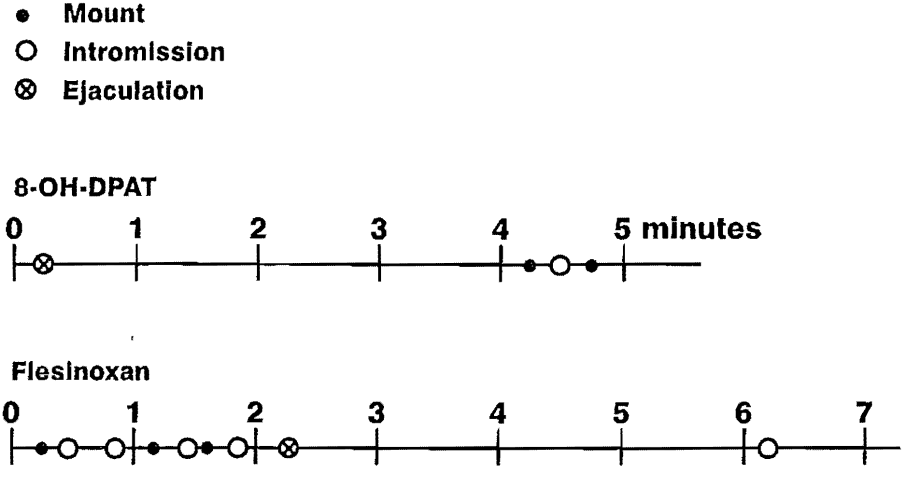

FIGURE 11.1. Typical pattern of male rat sexual behavior, influenced by the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT (cf. figure 3.2, page 25)

behavior and *ex copula* genital responses (e.g., Napoli-Farris *et al.*, 1984; Bitran and Hull, 1987). When testing male rat sexual behavior with a selective dopamine D_2 receptor agonist, like SND 919 (pramipexol) there is a significant decrease in the number of mounts and intromissions prior to ejaculation, and in ejaculation latency (Ferrari and Guiliani, 1994). However, none of the studies with dopaminergic compounds report ejaculations at the first or second intromission. The stimulatory properties of flesinoxan on both the 5-HT_{1A} autoreceptor and the D_2 receptor could work synergistically to decrease ejaculation latency, and the (limited) affinity for the dopamine D_2 receptor of flesinoxan could prevent male rats from ejaculating prematurely. Recently, 8-OH-DPAT is described to have a limited co-affinity for the 5-ht_7 receptor, which is mainly located in the rat hypothalamus (Sleight *et al.*, 1995). To our knowledge, no selective 5-ht_7 receptor agonists have been described.

Little pharmacotherapeutical options are available for men with ejaculatory difficulty (*ejaculatio retardata*) or the complete inability to ejaculate (anejaculation). In the case of undesired childlessness men are sometimes treated with transrectal electrostimulation of the prostate under general anesthesia to cause an ejaculation for the use of artificial insemination. On the basis of above described studies it is suggested to investigate if a 5-HT_{1A} receptor agonist, analogous to 8-OH-DPAT, might be an alternative treatment for these men.

Human studies with 5-HT_{1A} receptor agonists, like flesinoxan (8-OH-DPAT is not available for human studies) are required to assess the effects on sexual function in men and women. On the basis of the above described animal studies it could be hypothesized that flesinoxan could have a stimulating effect on sexual arousal in the human male.

In Part 2 the human studies are described. Premature ejaculation is not associated with penile hypersensivity: men with erectile dysfunction with or without premature ejaculation have higher penile thresholds to vibrotactile stimulation to the penis. The serotonin reuptake inhibitors clomipramine and fluoxetine increase latency to ejaculation in men with premature ejaculation. A limitation of the studies presented in chapters 9 and 10 is the small number of subjects participating in the studies.

Clomipramine (25 mg, 12-24 hours before anticipated sexual activity) appears to be more effective in increasing ejaculation latency in men with premature ejaculation than fluoxetine (5-10 mg/day). More research is required to find an

explanation for this difference. In these future studies, different doses of clomipramine and fluoxetine should be compared, and a comparison has to be made between daily use and a "as needed" regimen. Theoretically, the ideal drug for treatment of a sexual dysfunction has the following properties: 1. noninvasive administration (e.g. orally), 2. effective shortly after administration, 3. no side-effects, 4. inexpensive.

Fluoxetine (5-10 mg *daily*) had no effect on nocturnal penile tumescence (NPT) in men with or without erectile/ejaculatory dysfunction. With clomipramine (25 mg, once or twice *weekly*), we found a clear decrease of nocturnal penile circumference. Since fluoxetine and clomipramine are both serotonin reuptake inhibitors with similar potency (Benfield *et al.*, 1986), the present results were unexpected. Our findings corroborate with the results of Von Bardeleben and co-workers (1989). In a study of 6 healthy males, they found that a single dose of 80 mg fluoxetine did not alter the occurrence of nocturnal penile tumescence. An explanation for the differences between the effects of fluoxetine and clomipramine on NPT could be that clomipramine raises mean peak plasma prolactin (and cortisol) levels (Golden *et al.*, 1989). Cunningham and collaborators (1982) found an impairment of nocturnal penile tumescence in hyperprolactinaemic men.

Whether or not pharmacotherapy with serotonin reuptake inhibitors will be the treatment of choice in men with premature ejaculation, will depend on future studies on the long-term effects of these agents on sexual function, as well as on the conscientiousness of the patient taking the medication. Although most studies in the literature use daily administration of the drug, we prefer an "as needed" regimen. If with such a regimen the side-effects are too strong, one could change to daily treatment. These initial side-effects, like headache and dizziness, usually disappear after a few days when the medication is chronically taken.

Premature ejaculation is a condition with multiple modalities. There is a primary form and a secondary form. Intravaginal time to ejaculation is not the only important aspect, but it has also its repercussions on the partner, the relationship and on the self-image of the patient. It is unlikely that only a simple pill could solve all these problems. Careful history taking by a sexologically trained counseler, and a follow-up of patient and partner, possibly combined with the more traditional therapies, like the stop-start technique, are conditions for succesful treatment of premature ejaculation.

CONCLUSIONS

- 8-OH-DPAT renders male rats to become premature ejaculators
- 8-OH-DPAT doesn't make male rats more attractive than saline treated rats for an estrous female rat
- Testosterone is required for the stimulatory effects of 8-OH-DPAT on sexual behavior in male rats
- Flesinoxan stimulates sexual behavior in male rats without altering the pattern of this behavior and could be called a prosexual drug for male rats
- Premature ejaculation in humans is not caused by hypersensitivity of the penis
- Clomipramine (25 mg, taken as needed) effectively increases ejaculatory latency in men with premature ejaculation without erectile dysfunction. It also increases satisfaction with sex life and relationship in this group
- Fluoxetine (5-10 mg/ day) is effective in increasing latency to ejaculation in men with premature ejaculation

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Hoofdstuk 12

Samenvatting, discussie en conclusies

HET ONDERWERP VAN DEZE DISSERTATIE is om te onderzoeken welke rol de neurotransmitter serotonine (5-HT) en serotonine-achtige stoffen spelen bij de sturing van het ejaculatiemechanisme bij de rat en de mens. In *hoofdstuk 1* wordt een indeling gegeven, en de vragen die ten grondslag liggen aan de onderzoeken die in dit proefschrift staan beschreven worden gepresenteerd. Er zijn twee delen. In het eerste deel worden de rattenproeven beschreven waarbij twee vragen centraal staan. 1. Is het mogelijk om een rattenmodel te maken van de afwijking die we bij de mensen kennen als vroegtijdige zaadlozing (premature ejaculatie, *ejaculatio praecox*) met speciale serotonine verlagende stoffen in de hersenen (5-HT_{1A} receptor agonisten)? 2. Is het mogelijk het seksuele gedrag te stimuleren met deze serotonine verlagende stoffen bij ratten met lage seksuele activiteit?

In het tweede deel worden de humane onderzoekingen beschreven. De centrale vragen zijn: 3. Wordt vroegtijdige zaadlozing bij de mens veroorzaakt door een hypersensitiviteit (versterkte prikkelbaarheid) van de penis? 4. Wat is het effect van de antidepressiva clomipramine (Anafranil®) en fluoxetine (Prozac®), beide serotonine verhogende stoffen, op mannen met vroegtijdige zaadlozing, zonder of met erectiestoornissen en bij gezonde vrijwilligers?

In *hoofdstuk 2* wordt uitgelegd wat een neurotransmitter is. Een neurotransmitter, zoals serotonine, is een chemische stof die zorgt voor communicatie tussen zenuwcellen. Bij een electrische prikkel worden er in de overgangsspleet tussen twee zenuwcellen (synaps) een neurotransmitter vrijgemaakt, die door de volgende zenuwcel kan worden opgevangen door een speciale molecuul, de receptor. Als de receptor reageert met de neurotransmitter, dan wordt een nieuwe electrische prikkel gevormd, zodat het signaal over de rest van de zenuwcel kan worden doorgegeven. Er bestaan ook receptoren die de prikkel juist afremmen, zoals de serotonine-achtige 5-HT_{1A} receptor. Na de prikkeloverdracht worden de neurotransmitters afgebroken of heropgenomen in

de zenuwcel, zodat ze voor nieuwe prikkeloverdracht kunnen zorgen. Door stoffen te maken die lijken op die neurotransmitters, kunnen effecten worden nagebootst als de receptor met de stof kan reageren (agonist) of juist tegengaan als de receptor door de stof wordt geblokkeerd in zijn werking (antagonisten). Bij de rattenproeven worden 5-HT_{1A} receptor agonisten gebruikt (8-OH-DPAT en flesinoxan), die de hoeveelheid serotonine in bepaalde hersendelen verminderen. Bij de mensenproeven worden serotonine heropname remmers gebruikt, die er voor zorgen dat de hoeveelheid serotonine in de synaps hoog blijft, zodat er een extra lange en sterke prikkeloverdracht kan plaatsvinden.

Verder staat er in dit hoofdstuk een historisch overzicht van de indeling van de serotonine receptoren en worden er details gegeven van de stoffen die gebruikt zijn bij de verschillende experimenten.

DEEL 1

In *hoofdstuk 3* wordt allereerst het seksuele gedrag van de mannelijke rat beschreven. Grofweg bestaat dat uit beklimmingen (mounts), intromissies en ejaculaties. Een volwassen, ervaren mannelijke rat zal bij een bronstige vrouwelijke rat na enkele mounts ongeveer 6 of 7 intromissies nodig hebben voordat hij ejaculeert. Hierna volgt een periode van seksuele inactiviteit (post-ejaculatoire interval).

In het literatuuroverzicht wordt beschreven, dat 8-OH-DPAT het seksuele gedrag van de mannelijke rat ernstig kan ontwrichten. Niet alleen wordt de tijd tot ejaculatie verkort (van vijf minuten tot soms enkele seconden!), maar het copulatiepatroon verandert ook: zowel mounts als intromissies voor de eerste ejaculatie verdwijnen bijna geheel. Hierop is de analogie met de vroegtijdige zaadlozing bij de mens dan ook gebaseerd, waarbij soms de zaadlozing al plaatsvindt voordat er vaginale intromissie heeft plaatsgevonden. Verder wordt er een overzicht gegeven van andere stoffen, waarmee onderzoek is gedaan naar het seksuele gedrag bij mannelijke ratten, zoals serotonine (5-HT) zelf, flesinoxan (een 5-HT_{1A} receptor agonist) en ook dopamine, een andere neurotransmitter.

In de overige hoofdstukken van Deel 1 worden de experimenten beschreven die voor deze dissertatie in ons laboratorium zijn gedaan naar het effect van 5-HT_{1A} receptor agonisten op mannelijk seksueel gedrag bij de rat. In *hoofdstuk 4* worden enkele experimenten beschreven. In het eerste experiment vergelijken we

inactieve met actieve mannelijke ratten van "middelbare leeftijd" (12-14 maanden oud). Behandeling met 8-OH-DPAT stimuleert het seksuele gedrag bij deze ratten, en het verschil in de ejaculatiefrequentie, zoals dat bestond voordat ze met 8-OH-DPAT werden behandeld, verdwijnt.

In een volgend experiment wordt naar de ejaculatie gekeken bij ratten die behandeld waren met 8-OH-DPAT. Bij een dosis van 0.4 mg/kg ejaculeert 50-55% van de ratten bij de eerste of tweede intromissie ("premature ejaculatie"), en 25-35% van de mannelijke ratten ejaculeert buiten de vagina van het bronstige vrouwtje. Microscopische inspectie van het ejaculaat brengt geen abnormaliteiten aan het licht.

In een ander experiment wordt gekeken of een bronstig vrouwtje een met 8-OH-DPAT behandelde mannelijke rat misschien wel aantrekkelijker vindt dan een onbehandeld mannetje. Ze krijgt daartoe de keuze tussen een 8-OH-DPAT-man en een placebo-behandelde man in een drie-compartimenten kooi. Er blijkt geen verschil te zijn in tijd die ze doorbrengt met de twee mannelijke ratten, maar ze ontvangt meer ejaculaties van de 8-OH-DPAT man dan van de controlegroep. Verder brengt ze een toenemende hoeveelheid tijd door in het neutrale middencompartiment, waarin geen mannelijke rat aanwezig is.

Als een vrouwelijke rat bij een bronstige vrouwelijke rat wordt gezet, dan kan de eerste "mannelijk" beklimgedrag vertonen. In het laatste experiment wordt onderzocht of 8-OH-DPAT beklimgedrag bij vrouwtjes stimuleert. Alleen als de vrouwtjes chronisch met testosteron behandeld worden heeft 8-OH-DPAT een stimulerend effect op het masculine beklimgedrag.

Om uit te zoeken of ook bij mannelijke ratten een fysiologische hoeveelheid testosteron in het bloed nodig is om de effecten van 8-OH-DPAT op het seksuele gedrag te vinden, wordt een experiment uitgevoerd dat staat beschreven in *hoofdstuk 5*. Een groep van volwassen, seksueel ervaren mannelijke ratten wordt in een periode van een jaar regelmatig getest met een bronstig vrouwtje. Aanvankelijk gonadaal intact (gedurende 2 weken), daarna in gecastreerde toestand. Na 20 weken worden ze chronisch behandeld met een testosteron implantaat, zodat het hormoongehalte in het bloed weer genormaliseerd wordt. Het blijkt dat ongeveer 18 weken na castratie het niet meer mogelijk is om de intromissie en ejaculatie frequentie te stimuleren met 8-OH-DPAT. Dit vermogen keert echter wel terug nadat met de testosteron suppletie is gestart. Hiermee wordt aangetoond dat testosteron nodig is voor de stimulerende effecten van 8-OH-DPAT op het seksuele gedrag van de mannelijke rat.

In het laatste hoofdstuk van Deel 1, *hoofdstuk 6*, worden twee 5-HT_{1A} receptor agonisten, 8-OH-DPAT en flesinoxan vergeleken in hun effect op het seksuele gedrag van de mannelijke rat. Twee groepen ratten worden gebruikt: normale ratten en ratten die vlak na de geboorte behandeld zijn met een stof (ATD) waardoor op volwassen leeftijd o.a. het mannelijke seksuele gedrag geremd is. Met flesinoxan neemt het aantal ejaculaties per test toe en het aantal dieren dat dit gedrag gaat vertonen in beide groepen mannelijke ratten. Echter, er worden geen "premature ejaculaties" gezien (ejaculatie bij 1e of 2e intromissie). Daarom wordt in een tweede experiment 8-OH-DPAT vergeleken met flesinoxan. Wederom blijkt er dat met flesinoxan geen premature ejaculaties gevonden worden, terwijl met 8-OH-DPAT dat in beide groepen (ATD en controle mannetjes) wel gebeurt. Omdat flesinoxan wel de frequentie verhoogt maar niet het patroon aantast van seksueel gedrag bij mannelijke ratten, is het te beschouwen als een seksueel stimulerende stof voor mannelijke ratten. Daarentegen worden ratten "premature ejaculators" als ze met een adequate dosis 8-OH-DPAT worden behandeld.

DEEL 2

In het tweede deel van het proefschrift worden de humane onderzoekingen beschreven. Allereerst wordt er in *hoofdstuk 7* een overzicht gegeven van de literatuur over het onderwerp vroegtijdige zaadlozing en de behandeling daarvan met serotonine-achtige antidepressiva. Sinds de zeventiger jaren wordt er in de literatuur al melding gemaakt van seksuele bijwerkingen van antidepressiva die de heropname remmen van serotonine, zoals clomipramine en fluoxetine. Een van die bijwerkingen is het onvermogen om een orgasme te bereiken. Nadat dit bekend werd, zijn er vele studies gestart naar het effect van deze stoffen bij mannen met een vroegtijdige zaadlozing. De meeste onderzoeken tonen een gunstig effect aan voor deze stoffen, die de hoeveelheid serotonine in het centrale zenuwstelsel verhogen. Er is echter een tekort aan dubbel-blinde, placebo gecontroleerde crossover studies bij mannen met premature ejaculatie en gezonde vrijwilligers. Verder zijn er geen objectieve laboratoriumgegevens bekend over het effect op het seksuele functioneren van deze stoffen bij mannen met premature ejaculatie en/of erectiestoornissen en controles.

In *hoofdstuk 8* wordt onderzocht of mannen met vroegtijdige zaadlozing een sterkere gevoeligheid van de penis hebben dan mannen met erectiestoornissen of gezonde mannen zonder seksuele klachten. In het laboratorium wordt met behulp van een trilapparaatje aan de penis de drempelwaarde bepaald: welke trillingssterkte wordt nog wel wordt gevoeld, en welke niet meer. Het blijkt dat de drempelwaarden bij mannen met vroegtijdige zaadlozing en de controlegroep vergelijkbaar zijn, en dat mannen met erectiestoornissen met of zonder vroegtijdige zaadlozing een verhoogde drempelwaarde hebben. Mannen met premature ejaculatie alleen hebben dus geen verhoogde gevoeligheid, maar mannen met erectiestoornissen (met of zonder premature ejaculatie) hebben een verminderde gevoeligheid.

Een dubbel-blind, placebo gecontroleerd, crossover onderzoek naar het effect van clomipramine (Anafranil®) op het seksueel functioneren bij mannen met vroegtijdige zaadlozing en mannen zonder seksuele functiestoornissen wordt beschreven in *hoofdstuk 9*. Acht patienten met premature ejaculatie, 6 met premature ejaculatie en erectiele dysfunctie en 8 gezonde vrijwilligers nemen gedurende 3 weken 25 mg clomipramine 12-24 uur voor seksuele activiteit, gevolgd door 3 weken placebo (of andersom). Aan het begin en aan het einde van iedere periode bezoeken de proefpersonen het laboratorium. In het laboratorium wordt de seksuele functie gemeten bij de proefpersonen door de erectiele respons te meten tijdens een erotische video, zonder of met gelijktijdige stimulatie met een minivibrator aan de penis. Thuis wordt er een seksueel dagboek bijgehouden, en worden twee maal de nachtelijke erecties gemeten met een viltstof bandje (Erectiometer®). Met clomipramine neemt de tijd tot ejaculatie bij gemeenschap of masturbatie significant toe bij mannen met premature ejaculatie, maar niet bij mannen met premature ejaculatie plus erectiele dysfunctie of bij de controle mannen. In het laboratorium blijken mannen met premature ejaculatie na clomipramine inname beter in staat om hun ejaculatie te controleren. Verder rapporteren ze een verbetering van hun seksleven en van hun relatie. Tenslotte blijkt clomipramine bij alle proefpersonen verminderde nachtelijke erecties te geven.

In *hoofdstuk 10* wordt het laatste experiment van dit proefschrift beschreven. In een dubbel-blind, placebo gecontroleerd crossover onderzoek wordt het effect van de selectieve serotonine heropname remmer fluoxetine (Prozac®). onderzocht bij 9 mannen met premature ejaculatie, 9 met premature ejaculatie secundair aan erectiele dysfunctie, 7 met alleen erectiele dysfunctie en bij 15

gezonde vrijwilligers zonder seksuele problemen. Er zijn drie perioden van 4 weken: fluoxetine (5-10 mg/dag), geen behandeling en placebo (of omgekeerd). Het meten van de verschillende seksuele functies wordt op dezelfde manier verricht als bij de studie beschreven in hoofdstuk 9. De tijd tot ejaculatie neemt toe in de groep van mannen met premature ejaculatie plus erectiele dysfunctie en in de gecombineerde groep van mannen met premature ejaculatie met en zonder erectiele dysfunctie, maar niet statistisch significant in de groep met premature ejaculatie alleen. In het laboratorium stimuleert fluoxetine in alle groepen objectief maar niet subjectief de erectiele respons. Er zijn geen ongunstige bijwerkingen, en de nachtelijke erecties worden niet beïnvloed door fluoxetine.

DISCUSSIE

In het eerste deel van deze dissertatie wordt beschreven dat verschillende serotonine-achtige stoffen een effect hebben op het seksuele gedrag van de mannelijke rat. Met de stof 8-OH-DPAT, een 5-HT_{1A} receptor agonist die zorgt voor een lagere concentratie serotonine in het rattenbrein, blijkt het mogelijk een diermodel te maken voor de aandoening die we bij mensen kennen als vroegtijdige zaadlozing. Waar seksueel ervaren mannelijke ratten bij een bronstig vrouwtje na ongeveer zes of zeven intromissies ejaculeren, blijkt dat deze mannetjes onder invloed van 8-OH-DPAT al bij hun eerste of tweede intromissie ejaculeren. Dit wordt in verschillende experimenten, beschreven in de hoofdstukken 4-6, bestudeerd. Bij toekomstige proeven naar serotonerge sturing van seksueel gedrag bij mannelijke ratten verdient het aanbeveling om het effect te onderzoeken van serotonine heropname remmers bij ratten, die door behandeling met 8-OH-DPAT het copulatiepatroon van premature ejaculatie hebben gekregen. Een andere 5-HT_{1A} receptor agonist, flesinoxan, stimuleert wel het seksuele gedrag bij de mannelijke rat, maar tast het copulatiepatroon niet aan. Daarom is flesinoxan in de door ons gebruikte doses eerder als een seksueel stimulerende stof bij mannelijke ratten te beschouwen.

Het is onwaarschijnlijk dat serotonine de enige neurotransmitter is die voor sturing van seksueel gedrag bij de mannelijke rat zorgt. Enkele literatuur studies worden beschreven waarbij het effect van andere neurotransmitters op seksueel gedrag onderzocht wordt. Daaruit is geen eenduidig model te extraheren voor de sturing van ejaculatiegedrag in de mannelijk rat.

Voor mannen met moeite om te ejaculeren (*ejaculatio retardata*) of het totale onvermogen om te ejaculeren (anejaculatie) bestaan tot op heden weinig behandelingsmogelijkheden. In het geval van ongewenste kinderloosheid bij anejaculatie worden in geselecteerde gevallen mannen onder narcose gebracht om via transrectale electrische stimulatie aan de prostaat een ejaculatie op te wekken voor gebruik bij kunstmatige inseminatie. Op grond van de beschreven proeven met mannelijke ratten is het te overwegen om te onderzoeken of een 5-HT_{1A} receptor agonist, analoog aan 8-OH-DPAT, misschien een oplossing biedt.

Humane onderzoekingen met 5-HT_{1A} receptor agonisten, zoals bijvoorbeeld flesinoxan (8-OH-DPAT is niet beschikbaar voor humane toepassing) zijn nodig om het effect te bepalen op het seksuele functioneren bij mannen en vrouwen. Op grond van bovengenoemde dierexperimenten is het wellicht mogelijk dat flesinoxan een stimulerend effect op de seksuele opwinding heeft.

In het tweede deel van het proefschrift worden de humane studies beschreven. Bij mannen met vroegtijdige zaadlozing wordt eerst onderzocht of ze een hogere tactiele gevoeligheid van de penis hebben. Dit blijkt niet het geval. Verder blijkt dat de serotonine heropname remmers clomipramine en fluoxetine er voor zorgen dat mannen met premature ejaculatie een langere tijd tot ejaculatie hebben. Een beperking bij de beschreven studies is onder meer het bescheiden aantal proefpersonen dat participeert.

Het vermogen om de tijd te laten toenemen bij mannen met premature ejaculatie is sterker bij clomipramine (25 mg, 12-24 uur voor seksuele activiteit) dan bij een dagelijkse inname van 5-10 mg fluoxetine. Nader onderzoek is nodig om hiervoor een verklaring te vinden. Daarbij zullen verschillende doses van de genoemde stoffen moeten worden getest, en een vergelijking zal moeten worden gemaakt tussen een dagelijkse toediening en inname (kort) voor seksuele activiteit. In theorie heeft de ideale medicatie voor de behandeling van seksuele functiestoornissen heeft de volgende eigenschappen: 1. noninvasieve toediening (bv oraal), 2. inname kort voor of tijdens seksuele activiteit, 3. geen bijwerkingen, 4. goedkoop.

Of de serotonine heropname remmers de therapie van eerste keus zullen worden bij mannen met vroegtijdige zaadlozing, zal onder meer afhangen van toekomstig onderzoek, waarbij het lange-termijn effect van deze stoffen op de seksuele functie zal moeten worden onderzocht, evenals de therapietrouw op lange termijn. Een nadeel van deze serotonine-achtige stoffen bij gebruik vlak

voor seksuele activiteit (vergeleken met chronisch gebruik) is dat er initiële bijwerkingen kunnen blijven optreden, die meestal verdwijnen als de stof chronisch ingenomen wordt.

Vroegtijdige zaadlozing is een seksuele functiestoornis met meerdere aspecten. Zo is niet alleen de tijd tot ejaculatie bij seksuele activiteit van belang, maar heeft de aandoening bijvoorbeeld ook zijn repercussies op de partner, de relatie en op het zelfbeeld van de patient. Het is onwaarschijnlijk dat behandeling met uitsluitend een pil al deze problemen zal kunnen oplossen. Zorgvuldige intake door een seksuologisch geschoolde hulpverlener, en een intensieve begeleiding van patient en partner eventueel aangevuld met traditionele therapieën zoals stopstarttechniek zullen dan ook een voorwaarde blijven voor een succesvolle behandeling van vroegtijdige zaadlozing.

CONCLUSIES

• Toediening van 8-OH-DPAT bij de mannelijke rat veroorzaakt een seksueel gedrag dat equivalent is aan premature ejaculatie bij de mens

• Behandeling met 8-OH-DPAT maakt mannelijke ratten niet aantrekkelijker voor een bronstige vrouwelijke rat dan met placebo behandelde ratten

• Voor het stimulerende effect van 8-OH-DPAT op het seksuele gedrag van de mannelijke rat is een minimale hoeveelheid serum testosteron nodig

• Flesinoxan stimuleert het seksuele gedrag van de mannelijke rat zonder het patroon van het gedrag aan te tasten, en kan daarom een seksueel stimulerende stof voor mannelijke ratten worden genoemd

•Vroegtijdige zaadlozing bij de man wordt niet veroorzaakt door een verhoogde gevoeligheid van de penis

• Clomipramine (25 mg, 12-24 uur voor seksuele activiteit ingenomen) verlengt significant de tijd tot ejaculatie bij mannen met vroegtijdige zaadlozing zonder erectiestoornissen. Het verhoogt tevens de tevredenheid met het seksleven en met de relatie in deze groep

• Fluoxetine (5-10 mg dagelijks) verlengt de tijd tot ejaculatie bij mannen met vroegtijdige zaadlozing

List of abbreviations

ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
ATD	1,4,6-androstatriene-3,17-dione, an aromatase inhibitor
Contr	controls, comparison group
DALA	d-ala^2-met^5-enkephalinamide, a morphinomimetic agent
D receptor	dibenzyline-sensitive musculotropic receptor, a 5-HT subrecptor
DSM-IV	diagnostic and statistical manual of mental disorders, 4th edition
EB	estradiol benzoate
ED	erectile dysfunction
Ejac	ejaculations, ejaculation frequency
EL	ejaculation latency
FSH	follicle stimulating hormone
GABA	gamma-aminobutric acid, a neurotransmitter
5-HIAA	5-hydroxy-indoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
5-HTP	5-hydroxytryptophan, a 5-HT precursor
IL	intromission latency
Int	intromissions, intomission frequency
IP	intraperitoneal
L-DOPA	L-dihydrophenylalanine, a dopamine receptor agonist
LH	luteinizing hormone
LSD	least significant difference
MAOIs	monoamine oxidase inhibitors
mCPP	1-(m-chlorophenyl)piperazine, a 5-HT$_{1B}$ agonist
MDMA	3,4 methylenedioxymethamphetamine, ecstasy
ML	mount latency
M receptor	morphine-sensitive neurotropic receptor, a 5-HT subreceptor
Mts	mounts, mount frequency
n	number (of subjects)
NPT	nocturnal penile tumescence
NVVS	Nederlandse Vereniging voor Seksuologie
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin, a 5-HT$_{1A}$ receptor agonist
pCPA	p-chlorophenylalanine, a serotonin antagonist

PE	premature ejaculation
PEI	post-ejaculatory interval
PE/ED	premature ejaculation and erectile dysfunction
RU 24969	5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)indole, a 5-HT_{1B} receptor agonist
SC	subcutaneous
SME	simple main effects
SND 919	pramipexol, a selective dopamine D_2 receptor agonist
SSRI	selective serotonin reuptake inhibitor
TFMPP	1-(m-trifluoromethylphenyl)piperazine, a 5-HT_{1B} receptor agonist
TP	testosterone proprionate

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