Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 89 (2008) 209–217
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Sexual incentive motivation in old male rats: The effects of sildenafil and a compound (Impaza) stimulating endothelial NO synthase

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Received 4 October 2007; received in revised form 13 November 2007; accepted 11 December 2007
Available online 16 January 2008

Abstract

Several proerectile drugs act on the nitric oxide–cyclic guanosine monophopsphate pathway, which is known to influence rat copulatory behavior. In the present study we evaluated the effects of two proerectile compounds, one (Impaza) acting on endothelial nitric oxide synthase, and the other (sildenafil) on phosphodiesterase 5, on sexual incentive motivation in male rats displaying a spontaneously low level of motivation and copulatory behavior. About 20 months old male Fisher 344 rats were tested in a procedure for evaluating the intensity of sexual incentive motivation and in standard mating tests. For comparison, a group of young (about 4 months) Fisher 344 males was tested in parallel. This group did not receive any drug treatment. Impaza was administered in two doses, daily for 28 days, and sildenafil was given at a dose of 3 mg/kg twice a week during 28 days. Tests for sexual incentive motivation and copulatory behavior were performed immediately before the beginning of drug treatments, and on days 7, 14 and 28 of treatment. All treatment groups displayed a very low level of copulatory behavior and a sexually receptive female was not a more powerful incentive than another male at the tests performed before and on days 7 and 14 of treatment. On day 28 of treatment, the group treated with Impaza, 3 ml, displayed a preference for the sexually receptive female, while no such preference was found in the other groups. Furthermore, the preference score was above that of controls in this group. Both Impaza, 3 ml, and sildenafil reduced approach to the male in the test for sexual incentive motivation, suggesting that social motivation was reduced. These data suggest that compounds affecting the nitric oxide–cyclic guanosine monophopsphate pathway may modify both sexual and social motivation in old rats.

Keywords: Proerectile drugs; Sexual incentive motivation; Copulatory behavior; Aging rats; NO; PDE5; Sildenafil

1. Introduction

Sexual behavior is not possible at a distance. At least two individuals need to be in close proximity before any sexual interaction can take place. Thus, before sexual behaviors can be executed it is necessary to localize and approach a potential mate. The intensity of approach behaviors is generally believed to be determined by the intensity of sexual motivation (see Ågmo, 1999, 2003; Ågmo et al., 2004; Hetta and Meyerson, 1978; Meyerson and Lindström, 1973 for discussions of this issue). Research on the neural control of sexual motivation has become increasingly important because of the large amount of clinical data showing that low sexual desire is a human problem with an unexpectedly high prevalence (see e.g. Arnal et al., 1995; Laumann et al., 1999; Ventegodt, 1998). Consequently, the need for an efficient pharmacological treatment is widely recognized, but there is at present no treatment with established effects and there is no drug approved for the pharmacotherapy of hypoactive sexual desire disorder.

In men, erectile dysfunction may be associated with low sexual desire (Corona et al., 2004; Lewis et al., 2004), and it has been reported that treatment of the erectile deficiency may restore sexual desire (Jannini et al., 1999), suggesting that proerectile drugs indirectly may enhance sexual motivation. Recent data even suggest that some proerectile drugs may have a direct effect on motivational mechanisms. For example, it has been proposed that nitric oxide plays a role in the mechanisms involved in sexual motivation in addition to its well established importance for erection. An early study showed that the nitric

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doi:10.1016/j.pbb.2007.12.012

oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (NAME) reduced intromissions and ejaculation in male rats but enhanced mounting (Hull et al., 1994). This suggests that erection was adversely affected. In a test for sexual motivation, no effect was found. Different results have, however, been reported in more recent studies. When the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (NMMA) was administered to the medial preoptic area by reverse dialysis a reduced number of mounts was found. Interestingly, the intromission ratio (the proportion of mounts ending in vaginal penetration, intromission) remained unaffected (Sato et al., 1998). The intromission ratio is sensitive to changes in erectile function, and these data suggest that centrally reduced nitric oxide availability does not affect erectile capacity. Further evidence for a role of central nervous nitric oxide in male sexual behavior comes from a study in which NAME was infused into the medial preoptic area. Mounting was almost abolished in sexually inexperienced animals and severely reduced in animals with sexual experience (Lagoda et al., 2004). The results of these latter studies coincide in suggesting that nitric oxide is important not only for copulatory behavior but also for sexual motivation. The fact that many males did not copulate at all after treatment with NAME certainly suggests that sexual motivation was reduced or absent.

Many cellular actions of nitric oxide are dependent on the activation of guanylylcyclase and the subsequent formation of cyclic guanosinemonophosphate (cGMP). Nitric oxide responsive guanylylcyclase is widely distributed in the brain, including areas important for male sexual behavior (De Vente et al., 1998). It is, indeed, most likely that the effects on sexual behavior observed in the studies mentioned above are mediated by cGMP. Recent experimental data have confirmed this hypothesis (Sato and Hull, 2006).

Nitric oxide is also synthesized outside neurons, mainly through the action of endothelial nitric oxide synthase (eNOS). eNOS is present in blood vessels, including capillaries, and it has been shown that nitric oxide derived from eNOS diffuse into and affect adjacent neurons in a cGMP dependent way (Garthwaite et al., 2006). This makes it possible to envisage behavioral consequences of manipulations of eNOS. In fact, limited evidence for the importance of eNOS in sexual behavior comes from studies of mice lacking the gene for eNOS. Such mice ejaculate after fewer mounts and intromissions (Kriegsfeld et al., 1999). This suggests that the ejaculatory reflex was facilitated. No independent test for sexual motivation was performed.

cGMP is catabolized by a series of phosphodiesterases. One of these is phosphodiesterase 5. This enzyme is the target of several proerectile drugs, like sildenafil, tadalafil or zaprinast. The stimulatory effect of these drugs on erection is supposed to be localized to the corpora cavernosa (Uckert et al., 2006), but PDE5 has been found in several areas of the brain. Among the structures where the presence of PDE5 in large amounts has been established are the cerebellar Purkinje cells (Bender and Beavo, 2004). Several other brain areas, for example the olfactory bulbs and hippocampus express PDE5 to a much lesser degree (Lin et al., 2006). It is not impossible that this enzyme is also present and functionally relevant in brain areas related to sexual behavior. Concordant with this proposal is the fact that sildenafil affects several parameters of sex behavior when administered to male rats. In a group of males selected for unusually low intromission ratio and long intervals between intromissions the number of preejaculatory mounts, the ejaculation latency, and the postejaculatory interval were reduced while the intromission ratio was enhanced by sildenafil. In unselected rats, the only drug effect found was reduced ejaculation latency (Giuliani et al., 2002; Ottani et al., 2002). Sildenafil has also been shown to enhance inter-male mounting in sexually experienced but not in inexperienced rats (Ferrari et al., 2002). This last observation suggests that sildenafil has some stimulatory effect on sexual motivation. A study in rams also indicates that sildenafil may enhance the motivation to engage in copulatory behavior (Çoyan and Kaya, 2005). In this study sildenafil, at a dose of 100 mg per animal, was administered intrarectally to rams with low or high sexual activity according to performance on a screening test. Sildenafil enhanced the number of ejaculations in both groups. This effect was interpreted as suggestive of increased sexual motivation.

The data presented in the preceding paragraphs suggest that eNOS or nNOS (neural nitric oxide synthase) activated cGMP may influence male sexual behavior. Consequently, compounds modifying the activity of eNOS or nNos as well as phosphodiesterase inhibitors should be effective. Considerable evidence exists for a role of nNOS (see above), but data with regard to the role of eNOS in male sexual behavior are far from abundant. Likewise, the effects of phosphodiesterase inhibition, particularly inhibition of PDE5, have only been the subject of a handful of studies. More data with regard to the potential role of eNOS and PDE5 inhibition are needed before any firm conclusion as to their role in male sexual behavior can be drawn. The purpose of the present experiment is to contribute to that end. We evaluated the motivational effects of a compound, Impaza, acting on eNOS. Impaza is an antibody to the C-terminal fragment of eNOS, but paradoxically it has been reported to enhance the activity of endogenous eNOS when administered at extremely low doses. The compound is effective as monotherapy for erectile deficiency in the human and it also increases the efficacy of PDE5 inhibitors on combined treatment (Martyushev-Poklad et al., 2005; Mazo et al., 2004). Its effects on sexual motivation and copulatory behavior are entirely unknown. In order to determine possible motivational effects of PDE5 inhibition, one group of rats was treated with sildenafil. Both compounds were evaluated in a procedure especially designed for the evaluation of sexual incentive motivation (Ågmo, 2003; Ågmo et al., 2004). In addition, effects on copulatory behavior were determined in standard mating tests.

It is generally believed that male rats with low sexual activity are more sensitive to stimulatory actions of drugs than rats with high sexual activity. This belief was confirmed in the study of sildenafil mentioned above (Giuliani et al., 2002). Thus, in order to maximize the possibility of detecting potential effects of the treatments employed in the present experiment we used rats with low sexual activity. Rather than selecting a subsample of rats with such activity from a larger pool, we decided to use rats whose "normal" sexual activity is low. It is known that rats older than 20 months display much reduced sexual activity (Clark,

1995; Roselli et al., 1993; Smith et al., 1992; Tsai et al., 1994). Thus, we used rats around 20 months of age.

2. Materials and methods

2.1. Subjects

Male Fisher 344 rats were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN) through the Aged Rodent Colonies of the National Institute on Aging. The experimental males were 18 months old when arriving to the laboratory, and about 20 months old when drug treatments were initiated. Twelve additional Fisher 344 rats from the same provider were about 3 months old when arriving to the laboratory and about 4 months of age when behavioral observations were began. The young animals were included for comparison only, and they did not receive any drug treatment.

All subjects were housed in pairs in Macrolon cages under a reversed light/dark cycle (12:12 h, lights on 2300) in a room with controlled temperature (21±1 °C) and relative humidity (55±10%). Rodent pellets (RM1(E), Special Diet Services, Witham, Essex, UK) and tap water were freely available.

Male (300 g upon arrival) and female (250 g upon arrival) Wistar rats (Scanbur, Sollentuna, Sweden) were used as incentive animals in the test for sexual motivation. Similar females were used as copulation partners. These males and females were housed in the same room and under the same conditions as the experimental males.

All females were ovariectomized under isoflurane anesthesia at least 2 weeks before use. Before all testing sessions, estrus was induced by administration of estradiol benzoate, 25 μg/rat, followed by progesterone, 1 mg/rat, 48 h later. Females were used between 4 and 8 h after the progesterone injection. Both steroids were purchased from Sigma (St. Louis, MO, USA). They were dissolved in peanut oil and injected subcutaneously in a volume of 0.2 ml/rat.

The experimental procedures employed were approved by the Norwegian Committee for Ethics in Research on Animals and were in agreement with the European Union council directive 86/609/EEC.

2.2. Apparatus

Sexual incentive motivation was evaluated in a rectangular arena (100×50×45 cm high) with rounded corners. At the long sides were two diagonally opposed openings (25×25 cm). On the outside of each of these openings a small (15×25×25 cm high) box containing an incentive rat could be fitted. The animal inside the cage was separated from the arena by a double wire net. The mesh size was 12×12 mm and the two nets were separated by 10 mm. This meant that the animals could hear, see and smell each other while no direct physical contact was possible. Video cameras, fixed to the ceiling above the center of each arena, were connected to a computer and a videotracking system (Ethovision Pro, Noldus, Wageningen, the Netherlands) determined the experimental subjects' position with a frequency of 5 Hz. A virtual zone of 21×29 cm was defined outside each of the openings in the arena wall, and the time spent in these zones and the number of entries into them were determined. In addition, the distance moved during the test, the mean velocity of movement while moving and the time not moving were calculated. A more detailed description of the testing environment can be found in Ågmo (2003), and Ågmo et al. (2004).

Copulatory behavior was observed in rectangular sheet steel cages (40×60×40 cm high) with a Plexiglas front and glass floor. Under the floor there was a mirror tilted in an angle of 45°. This allowed for a ventral view of the experimental subjects. All test sessions were videotaped for later analyses.

The test for sexual incentive motivation was performed in a room lit by dim, white light. Light intensity at the level of the arena floor was about 5 lx. Tests for copulatory behavior were performed in an adjacent room. Here, the light intensity was around 25 lx, as measured at floor level in the mating cage.

2.3. Drugs

Sildenafil citrate was obtained as commercial tablets (Viagra®, Pfizer, USA) containing 25 mg. The tablets were crushed in a mortar and then dissolved in physiological saline. The reason for using commercial tablets rather than the pure compound was that we wanted to make the drug treatment as similar to the clinical use of sildenafil as possible. Antibodies to C-terminal fragment of endothelial NO synthase (mixture of homeopathic dilutions C12, C30, C200; Impaza®, OOO NPF Materia Medica Holding, Moscow, Russia) was provided as a ready-to-use solution in distilled water. The actual concentration of antibodies is not known, but the solution used here is identical to the one employed in clinical practice.

2.4. Design and procedure

The males were familiarized to the motivation test arenas during 3 sessions of 10 min each separated by 24 h. One of the incentive animal boxes contained a sexually receptive female and the other an intact male. The incentive animals were drawn from a lot of 10–12 rats maintained for the purpose, and were sexually experienced. This means that each incentive animal was used more than once. A few days after familiarization to the motivation test, screening tests for copulatory behavior were initiated. Up to this point, all animals were sexually naïve. At each test, the male was placed in the testing cage 5 min before the introduction of a sexually receptive female. The latency to the first mount with pelvic thrusting and the latency to the first vaginal penetration (intromission) as well as the number of mounts and intromissions before the first ejaculation were recorded. The ejaculation latency (time from the first intromission until ejaculation) and the postejaculatory interval (time between ejaculation and the next intromission) were also determined. The test was ended at the end of the postejaculatory interval, or 15 min after the introduction of the female if no intromission occurred, or 30 min after the first intromission if ejaculation had not occurred, or 15 min after ejaculation if no postejaculatory intromission was performed before that time. These tests were repeated twice weekly for 5 weeks. The same

criteria for ending the test were employed also at the tests performed during drug treatment. The likelihood of initiation of copulatory behavior after the first 10 min exposure to a female is very low (see e.g. Ågmo, 1999), so a longer test would probably not increase the proportion of sexually active males.

At the end of the training period, the subjects were randomly assigned to one of four groups of 10 rats each. One group was treated with distilled water, 2 ml/rat and day for 28 days. The second and third groups were given Impaza, 1 and 3 ml/rat and day, respectively, for 28 days. The fourth group was treated with sildenafil, 3 mg/kg in a volume of 2 ml/kg, twice weekly for 28 days. On the other days, these animals received distilled water, 2 ml/kg. All treatments were given orally by gavage. Care was taken to avoid leakage from the mouth. Again, oral treatment was used in order to be as close as possible to the clinical use of the compounds. This was also the rationale for giving sildenafil twice weekly rather than daily. Most men using sildenafil belong to an age group where the frequency of intercourse rarely exceeds twice a week. The sildenafil dose is within the range of doses that previously has been found to be effective in studies of copulatory behavior in young rats (Ferrari et al., 2002).

The experimental phase started with a 10 min test for sexual motivation immediately followed by a test for copulatory behavior. The following morning, drug administration was started. Tests were then performed at days 7, 14 and 28 of drug treatment. In parallel to the 4 experimental groups, a group of 12 young rats was tested for sexual incentive motivation and copulatory behavior in exactly the same way as the experimental rats. The young animals had been subjected to familiarization to the motivation arena and screening for copulatory behavior together with the experimental rats.

2.5. Statistics

Sexual motivation was quantified in several ways. Most important for evaluating the sexual incentive value of the receptive female is the preference score (time spent in the female incentive zone/(time spent in the female incentive zone+time spent in the male incentive zone)). The preference score at the pretest was analyzed with ANOVA with treatment as factor. The score obtained at day 28 of treatment was evaluated with an analysis of covariance with the preference score at pretest as covariate. With regard to the time spent in the female and male incentive zones as well as the number of visits to them, the pretest data were evaluated by two-factor ANOVAs with repeated measures on one factor, the within group factors being incentive (male, female) and the between-groups factor being treatment. Data from each of the tests performed during the treatment period were evaluated with an analysis of covariance with treatment as between-groups factor, incentive as within-groups factor and pretest time spent in the male and female zones as covariates. In case of significant interaction, tests for simple main effects of incentive within each treatment as well as effects of treatment within each incentive were performed (Winer et al., 1991). Indices of ambulatory activity were analyzed as the preference score.

Because of the low sexual activity displayed during the experiment, the analyses of the data from the copulatory behavior tests had to be limited to the proportion of subjects displaying mount, intromission or ejaculation. Treatments were compared with the chi-square test.

Data from the young group was not included in any of the abovementioned analyses. However, data obtained at the session immediately before the initiation of drug treatment to the experimental rats (pretest) were used for comparing the young group with the older rats. The *t*-test was employed for the analysis of the preference score and for all indices of ambulatory activity. The time spent in the incentive zones as well as the number of visits to them were evaluated with a mixed two-factor analysis of variance. The within-groups factor was incentive (male, female) and the between-groups factor was age (young, old).

Pretest data were also used for comparing copulatory behaviour in young and old rats. The proportion of animals displaying mount, intromission and ejaculation was analyzed with the Fisher exact probability test. The number of mounts and intromissions was evaluated with Mann–Whitney's *U*-test. The reason for employing a non-parametric test was that most animals had a value of 0 on both behavior patterns, making the distribution of the data dramatically skewed. The latencies and the postejaculatory interval were obtained from so few animals that a meaningful analysis was impossible.

3. Results

One animal in the group to be treated with Impaza, 1 ml, died before the beginning of drug administration. The same occurred to a rat in the group to be given Impaza, 3 ml. The local veterinarian performed autopsy but failed to identify the causes of death. It was attributed to old age. Two other animals lost weight and showed signs of bad health before the last drug treatment test. One belonged to the group given Impaza, 1 ml, and another to the group given sildenafil. These animals were eliminated from all analyses.

3.1. Sexual motivation

3.1.1. Pretest

There were no significant group differences in preference score at the pretest ($F_{3,32}=0.75$, NS). Likewise, there was no group difference in time spent in the incentive zones ($F_{3,32}=0.56$, NS), no difference between incentives ($F_{1,32}=0.23$, NS) and no interaction incentive × treatment ($F_{3,32}=0.99$, NS). Furthermore, none of the groups spent more time in the receptive female incentive zone than in the male incentive zone or had a preference score significantly above chance level. Data are illustrated in Fig. 1 A and B.

The number of visits to the incentive zones did not differ between groups ($F_{3,32}=0.92$, NS) or between incentives ($F_{1,32}=1.45$, NS). The interaction between group and incentive was also nonsignificant ($F_{3,32}=0.44$, NS). These data are not shown. The results from the pretest establish that the experimental groups did not differ significantly before the beginning of drug treatment.

When the pretest data from young and old rats were compared, it was found that the preference score was significantly

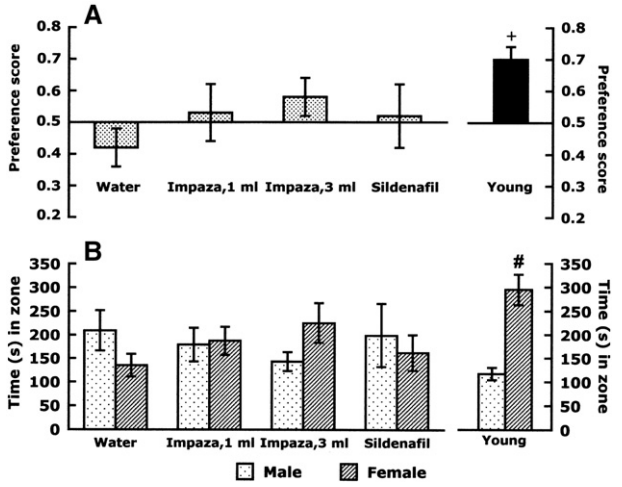

Fig. 1. A. Preference score in old male rats (left panel) obtained at the test performed before the beginning of drug treatments, the pretest. The right panel shows data from a group of 4 months old rats tested in parallel with the experimental groups. B. Time spent in the male and female incentive zones at the pretest in the experimental groups (left panel) and in a group of young rats (right panel). Data are mean±SEM. +, different from no preference, i.e. a score of 0.5, $P<0.05$. #, different from the male incentive, $P<0.05$. For further details, see text.

larger in young than in old rats ($t_{46}=2.64$, $P<0.05$). Furthermore, the score in the young rats was significantly above chance ($t_{11}=5.43$, $P<0.001$), showing that these animals preferred the female over the male (see Fig. 1A, right panel). Concerning the time spent in the incentive zones it was found that there was no main effect of age ($F_{1,46}=2.25$, NS). The main effect of incentive ($F_{1,46}=7.19$, $P=0.01$) as well as the interaction age × incentive ($F_{1,46}=8.50$, $P<0.01$) were significant. Tests for simple main effects of group within each incentive revealed that there was no difference between young and old rats with regard to the time spent in the male incentive zone ($F_{1,46}=2.83$, NS) while there was a difference in the time spent in the female incentive zone ($F_{1,46}=12.72$, $P=0.001$). Tests for simple main effects of incentive within groups showed that the young rats spent more time in the female than in the male incentive zone ($F_{1,46}=10.45$, $P<0.01$). There was no difference between incentives in the old rats ($F_{1,46}=0.06$, NS). Data are illustrated in the right panel of Fig. 1 B. Analysis of the number of visits to the incentives showed that the young rats made more visits than the old rats ($F_{1,46}=7.66$, $P<0.01$). There was no difference between the number of visits made to the male and female incentives ($F_{1,46}=2.60$, NS) and no interaction age × incentive ($F_{1,46}=0.08$, NS). Thus, the young rats were generally more active than the old rats.

The comparisons between young and old rats show that age did not influence approach to a social incentive (the male). In contrast, approach to a sexual incentive was much reduced in the old rats. Thus, sexual incentive motivation appears to be low or absent in such rats.

3.1.2. Tests during drug treatment

Since the young rats did not receive any drug treatment, no further mention is made of them. Consequently, the subsequent part of the results refers exclusively to data from the 20 months old rats.

The results from the tests at day 7 and 14 of treatment were similar to those obtained at the pretest. There was no significant difference between treatments, and the subjects did not show any preference for the sexually receptive female (all Ps<0.13, data not shown). On test day 28, there was a significant difference between treatments with regard to the preference score, ($F_{3,31}=3.93$, $P<0.05$). When treatment groups were compared to control, it turned out that the groups treated with Impaza, 3 ml, and sildenafil had a preference score above that of the control group ($P<0.05$). When the preference score obtained at each treatment was compared to no preference (a score of 0.5), it was found that only the group given Impaza, 3 ml, had a significant preference for the female incentive. Results are illustrated in Fig. 2A.

Analysis of the time spent in the incentive zones did not reveal any main effect of treatment ($F_{3,30}=0.15$, NS) or of incentive ($F_{1,30}=2.67$, NS) while the interaction treatment × incentive turned out to be significant ($F_{3,30}=4.08$, $P<0.05$). Tests for simple main effect of incentive within treatment showed that the subjects treated with Impaza, 3 ml, spent more time in the female incentive zone than in the male incentive zone ($F_{1,31}=10.53$, $P<0.01$). There was no significant difference between incentives within other treatments (Ps>0.08). When treatment effects within each incentive were analyzed, it turned out that the treatments differed with regard to the time spent in the male incentive zone ($F_{3,31}=4.14$, $P<0.05$) but not with regard to that spent in the female incentive zone ($F_{3,31}=1.23$, NS). *Post hoc* analyses revealed that the groups treated with Impaza, 3 ml, and sildenafil spent less time in the male incentive zone than

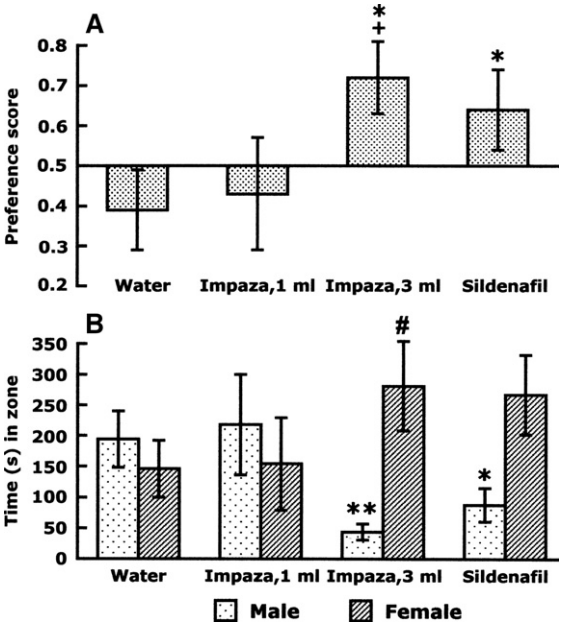

Fig. 2. A. Preference score in old male rats obtained at the test on day 28 of drug treatment. B. Time spent in the male and female incentive zones at day 28 of treatment. Data are mean±SEM. *, different from water, $P<0.05$; **, $P<0.01$. +, different from no preference (a score of 0.5), $P<0.05$. #, different from male, same treatment, $P<0.05$.

controls, while there was no effect of the other treatments (Fig. 2B).

3.2. Ambulatory activity

Indices of general activity revealed that the groups moved a similar distance during the pretest ($F_{3,32}=0.24$, NS), had a similar velocity of movement while moving ($F_{3,32}=0.27$, NS) and spent about the same time not moving ($F_{3,32}=0.33$, NS) at that test. This was also the case at the test performed on day 28 of treatment (distance, ($F_{3,32}=1.42$, NS; velocity ($F_{3,32}=0.86$, NS); inactivity time ($F_{4,40}=0.62$, NS)). Thus, no treatment affected any of the parameters indicative of general activity. Data from the test performed on day 28 of treatment are shown in Fig. 3.

While the drug treatments failed to affect ambulatory activity, data from the pretest show that age had a profound effect. Comparison between the young and old animals with regard to the distance moved during the test shows that the young moved longer than the old animals ($t_{46}=6.63$, $P<0.001$). Likewise, the young animals moved faster as revealed by analysis of the mean velocity of movement while moving ($t_{46}=3.15$, $P<0.01$). In contrast, there was no difference between young and old rats with regard to the time spent without moving ($t_{46}=1.27$, NS).

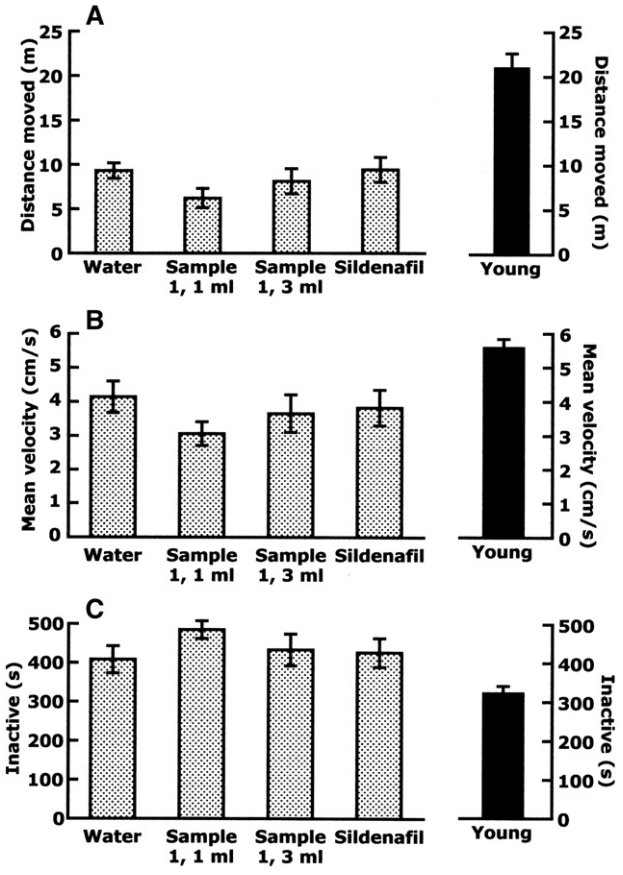

Fig. 3. Distance moved during the test performed at day 28 of treatment (A), mean velocity of movement (B) and time spent in inactivity (C) at that test. For comparison, data from a group of young rats tested in parallel with the experimental groups are shown to the right in each panel. Data are mean±SEM.

Table 1
Sexual behavior in male Fisher 344 rats about 4 months (Young) and about 20 months (Old) of age

Behavior	Young	Old
Proportion of animals displaying mount	58	8**
Proportion of animals displaying intromission	33	8
Proportion of animals displaying ejaculation	25	8
Number of mounts	8.1±3.3	1.2±0.78***
Number of intromissions	1.8±0.9	0.5±0.3*
Mount latency	145±70	80±51
Intromission latency	112±82	245±167
Ejaculation latency	487±95	453±105
Postejaculatory interval	430±32	392±86

Data are mean±SEM. Latencies are expressed in s. **, different from young, $P<0.01$; ***, $P<0.001$.

These data show that the younger rats moved a longer distance because they moved faster than the old rats but not because they spent more time moving. Data are illustrated in Fig. 3, right panels.

3.3. Copulatory behavior

All experimental groups displayed a very low level of sexual behavior at the pretest. In fact, the number of rats displaying mount, intromission and ejaculation was so low that no meaningful analysis of these parameters could be performed. Despite the fact that the rats were randomly assigned to the treatment groups immediately before the pretest, it appeared that the group to be treated with water included more sexually active animals than the other groups. During the entire experiment, 3 animals in this group mounted, intromitted and ejaculated on at least one occasion. In the group given sildenafil, one rat achieved ejaculation at the test on day 14 of treatment. Otherwise no copulatory activity was observed in any group. At the test performed on day 28 of treatment, for example, one rat in the group treated with water mounted, intromitted and ejaculated. In the other groups, not one single animal mounted. Not surprisingly, the χ^2 test revealed that there was no significant group difference (all Ps >0.34).

When the pretest data from the young rats were compared to those from the old rats several differences were found. The proportion of young rats displaying at least one mount was significantly larger than that of the old rats ($P=0.001$, the Fisher test), and the number of mounts performed was superior in the young rats (Mann–Whitney's $U=112$, $P<0.001$). There was a borderline effect of age on the proportion of rats displaying intromission ($P=0.051$, the Fisher test) while the number of intromissions was larger in the young than in the old rats (Mann–Whitney's $U=166.5$, $P<0.05$). To the contrary, there was no significant difference with regard to the proportion of animals ejaculating ($P=0.15$, the Fisher test). This is due to the rather low number of young rats achieving ejaculation at this particular test. The low number of old rats displaying copulatory behavior made statistical comparisons of mount, intromission and ejaculation latencies as well as of the postejaculatory interval meaningless. Nevertheless, it can be maintained that about 4 months old Fisher 344 rats display a more intense sexual

behavior than rats 20 months old do. Data are summarized in Table 1.

4. Discussion

The Fisher 344 rats displayed very little sexual behavior when their first encounter with a sexually receptive female occurred at the age of 20 months. In fact, their sexual behavior was much inferior to that previously reported for Fisher rats having acquired extensive sexual experience when young. When tested at about the same age (21–22 months) between 30 and 40% of these rats displayed ejaculation (Chambers et al., 1991; Roselli et al., 1993). Substantial sexual activity has also been found in old, sexually experienced rats of other strains (Smith et al., 1992). The proportion of rats displaying ejaculation at pretest in the present study, 6%, was much lower than in the studies mentioned above. This observation suggests that acquisition of sexual experience when young enhances the likelihood of displaying sexual behavior when old. Indeed, the only other study in which sexually inexperienced animals were employed reported that only 16% ejaculated when tested at 18–19 months of age (Tsai et al., 1994). This is not much different from the results obtained with our 20 months old animals, supporting the notion that prior experience is a crucial determinant of sexual behavior in old rats. Independently of this, it can be concluded that our aim of testing Impaza and sildenafil in rats with spontaneously low sexual activity was fulfilled.

Not only did most of the old rats fail to display sexual behavior, but they also failed to show any signs of sexual incentive motivation. They did not spend more time in the vicinity of a receptive female than in the vicinity of another male. This is in sharp contrast to a wealth of data from young male rats, in which a preference for the receptive female has been solidly established in a variety of procedures (see Ågmo et al., 2004; Pfaff and Ågmo, 2002, for a discussion). It is probably so that the sexually receptive female's lack of incentive properties for old males is the cause of the absence of copulatory behavior in these males. As pointed out in the Introduction, sexual behavior is not possible at a distance, and if the female is unable to activate approach behaviors there is no way she can activate copulatory behaviors. Sexual incentive motivation was not evaluated in any of the studies with old rats mentioned above, but it is likely that the reduced proportion of males displaying copulatory behavior as well as the reduced intensity of that behavior in the few males who did display it are a consequence of reduced sexual motivation. The data from the group of young rats tested in parallel to the experimental subjects substantiate this notion. The young animals did not only show a more intense sexual incentive motivation than the old animals but also a more intense copulatory behavior. It seems, then, that old male rats constitute a good model for studying potential treatments for reduced or absent sexual incentive motivation.

Impaza, 3 ml, seemed to enhance sexual incentive motivation at the test performed on day 28 of treatment in the way that the experimental males approached the incentive female more than the incentive male. However, this was mainly due to a reduced intensity of approach to the male incentive. The time spent in the female incentive zone was increased compared to control, but not sufficiently for statistical significance, making it questionable whether the female's incentive value was enhanced by the drug treatment or not. Indeed, the continued absence of copulatory behavior shows that the incentive motivational properties of the female were not sufficient for making the males engage in copulatory activity. Nevertheless, it is possible that a longer treatment period would have succeeded in having more robust effect on incentive motivation and eventually also on copulatory behavior. Since the time course of the effects of Impaza on eNOS is not well known, this is pure speculation, however. In this context it is worthwhile to remember that much evidence show that approach to a potential mate is controlled by mechanisms partly different from those controlling the execution of copulatory behavior (see e.g. Ågmo, 2002), and that male rats may display approach behavior even though copulation does not follow (Stone et al., 1935). To the contrary, copulation without preceding approach is impossible, as already pointed out.

Sildenafil did not significantly modify sexual incentive motivation although its effect on the preference score was of borderline significance ($P=0.05$). Like Impaza, 3 ml, it reduced the time spent in the male incentive zone at the test performed on day 28 of treatment without producing any significant increase in the time spent in the female incentive zone. This observation suggests that the effect seen with Impaza, 3 ml, is not spurious but somehow related to enhanced activity of NO-dependent mechanisms. How such enhanced activity reduces approach to the male incentive is not entirely clear, but some speculations can be made. We have earlier shown that approach to the male is mainly determined by social motivation. For example, manipulations altering sexual motivation, like castration or extensive sexual activity immediately preceding the test, do not modify approach behaviors to the male (Ågmo, 2003; Ågmo et al., 2004). Others have also shown that male rats approach other males (Eckman et al., 1969; Latané, 1969; Latané et al., 1972, 1973; Latané and Glass, 1968), and that this social approach is independent of immediately preceding sexual activity (Sloan and Latané, 1974). In view of this it is likely that the reduced approach to the male seen after Impaza, 3 ml and sildenafil is a result of lowered social motivation.

This proposal is substantiated by the fact that the time spent in the male incentive zone after Impaza, 3 ml and sildenafil is not larger than the time the experimental subjects would have spent there if their position in the arena were random. A calculation of the time the males should be expected to spend in the incentive zones if their position indeed were random gives 83 s ((incentive zone surface/total arena surface)×test duration). This is superior to the value obtained in the group treated with Impaza, 3 ml, in which the experimental males spent 44±13 s (mean±SE) in the male incentive zone. In fact, this value is significantly different from the expected value of 83 s ($t_8=2.89$, $P<0.05$). It appears, then, that the males treated with Impaza, 3 ml actively avoided the incentive male. In the group given sildenafil there was no difference between actual and theoretical random time in the male incentive zone (88±27 and 83 s, respectively; $t_9=0.86$, NS), suggesting that these males neither approached nor avoided the male incentive. For comparison, it

may be mentioned that the control males spent more time in the male incentive area than random position would predict (194±46, $t_9=2.42$, $P<0.05$). This coincides with earlier data showing that male subjects spend more time in the vicinity of another male than in the vicinity of an empty incentive cage (Ågmo et al., 2004). Furthermore, this observation confirms that male rats are socially attracted to other males.

The arguments exposed in the preceding paragraph suggest that sildenafil abolished social motivation while Impaza, 3 ml, not only eliminated the incentive male's social incentive value, but also turned him into a negative incentive, producing withdrawal. These effects could, in principle, be explained by an anxiogenic action of Impaza and in minor degree of sildenafil. A study performed in male Fisher 344 rats shows that anxiogenic compounds reduce the time spent close to an inaccessible social incentive (a male rat) whereas anxiolytic compounds enhance it (Nicolas and Prinssen, 2006). Indeed, a reduced time close to a social incentive was exactly what was observed in the present experiment. However, data are contradictory with regard to the effects of nitric oxide and cGMP on anxiety (see Guimaraes et al., 2005 for a review). Some effects have been reported in the elevated plus-maze, but the few studies employing the social interaction test for anxiety have reported mixed effects of altered availability of nitric oxide. For example, Volke et al. (1997) reported enhanced social interaction following treatment with the nitric oxide synthase inhibitor 7-nitroindazole. However, a study employing another nitric oxide synthase inhibitor, NAME, did not find any effect at all on social interaction (Vale et al., 1998). The effects of manipulations of cGMP concentrations with sildenafil on anxiety-like behaviors have not been much studied, but the majority of data suggests an anxiogenic action (Kurt et al., 2004; Volke et al., 2003). Unfortunately, the data stem exclusively from mice, and there is no study with regard to effects on social interaction. Nevertheless, it does not seem unreasonable to suggest that the reduced social motivation observed after treatment with sildenafil or Impaza, 3 ml may be related to anxiogenic effects of enhanced activity in the nitric oxide–cGMP pathway. Additional experiments are needed in order to determine how nitric oxide-dependent mechanisms modify social motivation.

An interesting consequence of the reduced social motivation displayed by the animals treated with sildenafil and particularly with Impaza, 3 ml combined with the fact that the time spent in the female incentive area was not reduced, is that the female's sexual incentive value must have been enhanced by these treatments. Considering that the female is both a social and sexual incentive, reduced social motivation must have been compensated for by an increase in sexual motivation. Otherwise, the time spent in the female incentive area should have been reduced. Thus, it can be speculated that sildenafil and especially Impaza, 3 ml, enhanced the female's sexual incentive value. However, only further studies in other procedures can substantiate this proposal. Nevertheless, the data obtained in the experiment reported here are sufficiently suggestive to justify such studies.

None of the behavioral effects observed after treatments with sildenafil and Impaza can be attributed to altered general activity. All activity indices failed to detect any difference between treatments. It is noteworthy, though, that a group of younger Fisher 344 males had a much higher activity than the old males employed in the experiment. This observation suggests that reduced sexual activity is only one of many behavioral changes occurring with advancing age.

At present, the pharmacokinetics and pharmacodynamics of Impaza are entirely unknown. However, the data mentioned in the Introduction show that it does stimulate eNOS activity and that it constitutes an efficient proerectile treatment. Since eNOS is located in blood vessels, there is no need for the compound to cross the blood-brain barrier in order to be active. Nevertheless, it needs to be absorbed from the intestines and enter the circulation. The exact mechanisms participating in these processes are not known. Incomplete knowledge of the mechanisms of action is not unusual among clinically active compounds, though.

In sum, the data obtained in the present experiment show that old male rats fail to approach a sexually receptive female more than another male. Furthermore, sexually naïve old males show very little copulatory behavior when given access to a sexually receptive female. Stimulation of eNOS may enhance sexual incentive motivation in these old rats without activating copulatory behavior. The PDE5 inhibitor sildenafil had a borderline effect on incentive motivation and none on copulatory behavior. The effects on social motivation observed in the present experiment are difficult to explain, but they suggest that nitric oxide–cGMP dependent mechanisms are among the many central nervous mechanisms determining social incentive value. Present results show that proerectile compounds acting through the nitric oxide–cGMP system may have important effects on motivational processes.

Acknowledgements

The experiment reported herein was financed by OOO NPF Materia Medica Holding, Moscow, Russia. The technical assistance of Ragnhild Osnes and Stig Rune Olsen is gratefully acknowledged. The authors have no financial interest in OOO NPF Materia Medica Holding.

References

Ågmo A. Copulation-contingent aversive conditioning and sexual incentive motivation in male rats: evidence for a two-stage process of sexual behavior. Physiol Behav 2002;77:425–35.

Ågmo A. Sexual motivation. An inquiry into events determining the occurrence of sexual behavior. Behav Brain Res 1999;105:129–50.

Ågmo A. Unconditioned sexual incentive motivation in the male Norway rat (*Rattus norvegicus*). J Comp Psychol 2003;117:3–14.

Ågmo A, Turi AL, Ellingsen E, Kaspersen H. Preclinical models of sexual desire: conceptual and behavioral analyses. Pharmacol Biochem Behav 2004;78:379–404.

Arnal A, Llario R, Gil MD. Estudio epidemiológico sobre la prevalencia de disfunciones sexuales en la comunidad valenciana. Psicothema 1995;7:95–104.

Bender AT, Beavo JA. Specific localized expression of cGMP PDEs in Purkinje neurons and macrophages. Neurochem Int 2004;45:853–7.

Chambers KC, Thornton JE, Roselli CE. Age-related deficits in brain androgen binding and metabolism, testosterone, and sexual behavior of male rats. Neurobiol Aging 1991;12:123–30.

Clark JT. Sexual function in altered physiological states: comparison of effects of hypertension, diabetes, hyperprolactinemia, and others to "normal" aging in male rats. Neurosci Biobehav Rev 1995;19:279–302.

Corona G, Mannucci E, Mansani R, Petrone L, Bartolini M, Giommi R, et al. Aging and pathogenesis of erectile dysfunction. Int J Impot Res 2004;16: 395–402.

Çoyan K, Kaya A. Der Einfluss von Sildenafil (Viagra®) auf das Geschechtsverhalten von Schafböcken. Wiener Tierarztl Monatsschr 2005;92:30–3.

De Vente J, Hopkins DA, Markerink-Van Ittersum M, Emson PC, Schmidt HHHW, Steinbusch HWM. Distribution of nitric oxide synthase and nitric oxide-receptive, cyclic GMP-producing structures in the rat brain. Neuroscience 1998;87:207–41.

Eckman J, Meltzer JD, Latané B. Gregariousness in rats as a function of familiarity of environment. J Personal Soc Psychol 1969;11:107–14.

Ferrari F, Ottani A, Giuliani D. Influence of sildenafil on central dopamine-mediated behaviour in male rats. Life Sci 2002;70:1501–8.

Garthwaite G, Bartus K, Malcolm D, Goodwin D, Kollb-Sielecka M, Dooldeniya C, et al. Signaling from blood vessels to CNS axons through nitric oxide. J Neurosci 2006;26:7730–40.

Giuliani D, Ottani A, Ferrari F. Influence of sildenafil on copulatory behaviour in sluggish or normal ejaculator male rats: a central dopamine mediated effect? Neuropharmacology 2002;42:562–7.

Guimaraes FS, Beijamini V, Moreira FA, Aguiar DC, de Lucca ACB. Role of nitric oxide in brain regions related to defensive reactions. Neurosci Biobehav Rev 2005;29:1313–22.

Hetta J, Meyerson BJ. Sexual motivation in the male rat. A methodological study of sex-specific orientation and the effects of gonadal hormones. Acta Physiol Scand 1978;Suppl. 453:1–67.

Hull EM, Lumley LA, Matuszewich L, Dominguez J, Moses J, Lorrain DS. The roles of nitric oxide in sexual function of male rats. Neuropharmacology 1994;33:1499–504.

Jannini EA, Screponi E, Carosa E, Pepe M, Lo Giudice F, Trimarchi F, et al. Lack of sexual activity from erectile dysfunction is associated with a reversible reduction in serum testosterone. Int J Androl 1999;22:385–92.

Kriegsfeld LJ, Demas GE, Huang PL, Burnett AL, Nelson RJ. Ejaculatory abnormalities in mice lacking the gene for endothelial nitric oxide synthase (eNOS−/−). Physiol Behav 1999;67:561–6.

Kurt M, Bilge SS, Aksoz E, Kukula O, Celik S, Kesim Y. Effect of sildenafil on anxiety in the plus-maze test in mice. Polish J Pharmacol 2004;56:353–7.

Lagoda G, Muschamp JW, Vigdorchik A, Hull EM. A nitric oxide synthesis inhibitor in the medial preoptic area inhibits copulation and stimulus sensitization in male rats. Behav Neurosci 2004;118:1317–23.

Latané B. Gregariousness and fear in laboratory rats. J Exp Soc Psychol 1969;5:61–9.

Latané B, Glass DC. Social and nonsocial attraction in rats. J Personal Soc Psychol 1968;9:142–6.

Latané B, Edwards J, Steele C, Walton D. Social attraction among and between albino and hooded rats. Bull Psychonom Soc 1973;2:20–2.

Latané B, Joy V, Meltzer J, Lubell B, Cappell H. Stimulus determinants of social attraction in rats. J Comp Physiol Psychol 1972;79:13–21.

Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA 1999;281:537–44.

Lewis RW, Fugl-Meyer KS, Bosch R, Fugl-Meyer AR, Laumann EO, Lizza E, et al. Epidemiology/risk factors of sexual dysfunction. J Sexual Med 2004;1:35–9.

Lin CS, Lin GT, Xin ZC, Lue TF. Expression, distribution and regulation of phosphodiesterase 5. Curr Pharmaceut Design 2006;12:3439–57.

Martyushev-Poklad A, Mazo E, Gamidov S, Dugina J, Epstein O, Sergreeva S. Impaza, an oral antibody therapeutic for erectile dysfunction: benefits from mono-and combined therapy. In: Sae CK, Grootegoed JA, Chemes HE, editors. Proceedings of the VIIIth International Congress of Andrology. Bologna, Italy: Medimond; 2005. p. 49–54.

Mazo EB, Gamidov SE, Ovchinnikov RI. Viagra, Sialis, Impasa — which of them, to whom, when and how? Urologiia 2004;5:42–8.

Meyerson BJ, Lindström LH. Sexual motivation in the female rat. A methodological study applied to the investigation of the effect of estradiol benzoate. Acta Physiol Scand 1973;Suppl. 389:1–80.

Nicolas LB, Prinssen EPM. Social approach-avoidance behavior of a high-anxiety strain of rats: effects of benzodiazepine receptor ligands. Psychopharmacology 2006;184:65–74.

Ottani A, Giuliani D, Ferrari F. Modulatory activity of sildenafil on copulatory behaviour of both intact and castrated male rats. Pharmacol Biochem Behav 2002;72:717–22.

Pfaff DW, Ågmo A. Reproductive motivation. In: Pashler H, Gallistel R, editors. Steven's Handbook of Experimental Psychology. Volume 3, Learning, Motivation, and Emotion. New York: Wiley; 2002. p. 709–36.

Roselli CE, Thornton JE, Chambers KC. Age-related deficits in brain estrogen receptors and sexual behavior of male rats. Behav Neurosci 1993;107:202–9.

Sato SM, Hull EM. The nitric oxide-guanosine 3′,5′-cyclic monophosphate pathway regulates dopamine efflux in the medial preoptic area and copulation in male rats. Neuroscience 2006;139:417–28.

Sato Y, Horita H, Kurohata T, Adachi H, Tsukamoto T. Effect of the nitric oxide level in the medial preoptic area on male copulatory behavior in rats. Am J Physiol, Regul Integr Comp Physiol 1998;43:R243–7.

Sloan L, Latané B. Sex and sociability in rats. J Exp Soc Psychol 1974;10:147–58.

Smith ER, Stefanick ML, Clark JT, Davidson JM. Hormones and sexual behavior in relationship to aging in male rats. Horm Behav 1992;26:110–35.

Stone CP, Barker RG, Tomilin MI. Sexual drive in potent and impotent male rats as measured by the Columbia obstruction apparatus. J Genet Psychol 1935;47:33–48.

Tsai YF, Tsai HW, Tai MY. Variations in the sexual performance in old male rats. Zool Stud 1994;33:232–4.

Uckert S, Hedlund P, Andersson KE, Truss MC, Jonas U, Stief CG. Update on phosphodiesterase (PDE) isoenzymes as pharmacologic targets in urology: present and future. Eur Urol 2006;50:1194–207.

Vale AL, Green S, Montgomery AMJ, Shafi S. The nitric oxide synthesis inhibitor L-NAME produces anxiogenic-like effects in the rat elevated plus-maze test, but not in the social interaction test. J Psychopharmacol 1998;12:268–72.

Ventegodt S. Sex and quality of life in Denmark. Arch Sex Behav 1998;27: 295–307.

Volke V, Soosaar A, Koks S, Bourin M, Mannisto PT, Vasar E. 7-nitroindazole, a nitric oxide synthase inhibitor, has anxiolytic like properties in exploratory models of anxiety. Psychopharmacology 1997;131:399–405.

Volke V, Wegener G, Vasar E. Augmentation of the NO-CGMP cascade induces anxiogenic-like effect in mice. J Physiol Pharmacol 2003;54:653–60.

Winer BJ, Brown DR, Michels KM. Statistical principles in experimental design. New York: McGraw-Hill; 1991.