

# Effect of sildenafil (Viagra®) on the genital reflexes of paradoxical sleep-deprived male rats

M.L. Andersen,  
M. Bignotto and S. Tufik

Departamento de Psicobiologia, Escola Paulista de Medicina,  
Universidade Federal de São Paulo, São Paulo, SP, Brasil

## Abstract

### Correspondence

M.L. Andersen  
Departamento de Psicobiologia  
EPM, UNIFESP  
Rua Napoleão de Barros, 925  
04024-002 São Paulo, SP  
Brasil  
Fax: +55-11-5572-5092  
E-mail: mandersen@psicobio.epm.br

Research supported by  
Associação Fundo de Incentivo  
à Psicofarmacologia (AFIP) and  
FAPESP (No. 01/04329-0 to  
M.L. Andersen) and CEPID (No.  
98/14303-3 to S. Tufik).

The authors declare that they do not  
own stock of, participate in clinical  
research for, or belong to the  
speakers' bureau of the manufacturer  
of sildenafil (Pfizer, New York).

Received September 21, 2006  
Accepted August 30, 2007

Since there is evidence that paradoxical sleep deprivation (PSD) elicits penile erection (PE) and ejaculation (EJ), and that the erectile response of rats is mediated by nitric oxide, the present study sought to extend the latter finding by assessing the effects of sildenafil on the genital reflexes of male Wistar rats subjected to PSD. We also determined the influence of sildenafil on hormone concentrations. In the first experiment, sildenafil at doses ranging from 0.08 to 0.32 mg/kg was administered intraperitoneally to rats that had been deprived of sleep for 4 days and to home cage controls (N = 8-10/group). The frequency of PE and EJ was measured for 60 min. PSD alone induced PE in 50% of the animals; however, a single injection of sildenafil did not significantly increase the percentage of rats displaying PE compared to PSD-saline or to home cage groups. PSD alone also induced spontaneous EJ, but this response was not potentiated by sildenafil in the dose range tested. Testosterone concentrations were significantly lower in PSD rats ( $137 \pm 22$  ng/dL) than in controls ( $365 \pm 38$  ng/dL), whereas progesterone ( $0.9 \pm 0.1$  vs  $5.4 \pm 1$  ng/mL) and plasma dopamine ( $103.4 \pm 30$  vs  $262.6 \pm 77$  pg/mL) increased. These changes did not occur after sildenafil treatment. The data show that although sildenafil did not alter the frequency of genital reflexes, it antagonized hormonal (testosterone and progesterone) and plasma dopamine changes induced by PSD. The stimulation of the genital reflexes by sildenafil did not result in potentiating effects in PSD rats.

### Key words

- Paradoxical sleep deprivation
- Sildenafil
- Genital reflexes
- Dopamine
- Testosterone
- Progesterone

## Introduction

It has been suggested that nitric oxide (NO) is the primary physiological mediator of penile erection (PE) (1,2). Several investigators have shown that PE induced by electrical stimulation of the pelvic plexus cavernous nerve was prevented by inhibitors of NO synthase, which synthesizes the transmitter/modulator NO (3) in several tissues (4), including brain (5). It is generally ac-

cepted that NO originates in nonadrenergic noncholinergic nerve fibers of the penis, and is the principal vasodilator during the erectile response (6). This response is abolished by systemic and intracerebroventricular administration of NO synthase inhibitors (6).

Sildenafil citrate (Viagra®, Pfizer, New York, NY, USA) is an oral medication considered to be a first-line therapy for erectile dysfunction. It enables an erection in response to sexual stimulation through selec-

tive inhibition of cyclic GMP-specific phosphodiesterase type 5 (7). Erections occur through sexual stimulation of the parasympathetic nervous system leading to release of NO, a powerful vasodilator derived from the endothelial cells lining the corpus cavernosum. NO produces cyclic GMP, which causes the smooth muscle of the corpus cavernosum to relax and fill with arterial blood, thus producing the erection (8).

Sleep loss is associated with several behavioral responses, some of which are under hormonal control and include sexual behavior. We recently reported data showing that paradoxical sleep deprivation (PSD) elicited genital reflexes (penile erection and ejaculation) in male rats and that these behaviors were potentiated by a single injection of dopaminergic agents (9,10). Moreover, the study of genital reflexes induced in PSD rats in the absence of receptive females seems to be a good model for the penile erection response to different drugs such as cocaine (11,12). Specifically, the direct application of cocaine to the corpus cavernosum of the penis increases and prolongs intracavernous pressure in the rat, and the higher intracavernous pressure caused by cocaine has been hypothesized to be mediated by NO within the corpus cavernosum (13).

Based on findings that cocaine (11,12) and apomorphine, used as a therapeutic strategy for erectile dysfunction (13), potentiated genital reflexes in PSD rats (9), and further, that cocaine-induced penile erection is mediated by NO (14), on which sildenafil acts, the objective of the present study was to investigate, by administering sildenafil, whether the NO mechanism is involved in penile erection, ejaculation, and the hormonal changes promoted by PSD.

## Material and Methods

### Subjects

Three-month-old male Wistar rats from

our facilities were used in this experiment. The vivarium was maintained on a 12-h light/dark cycle (lights on from 7:00-19:00 h) at an ambient temperature of 22°C. Laboratory chow and water were provided *ad libitum*. All procedures used in the present study complied with the Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Ethics Committee of UNIFESP (CEP #206/02).

### Paradoxical sleep deprivation

The PSD method is based on the use of 14 narrow circular platforms (6.5 cm in diameter) placed inside a tilted tank (123 x 44 x 44 cm) filled with water up to within 1 cm from the rim of the platforms. The animals were placed on the top of the platforms and were able to move around by leaping from one platform to another. Each group was placed in one tank. When they reached the paradoxical phase of sleep, they fell into the water due to muscle atonia and woke up. When choosing this technique our intention was to evaluate genital reflexes that would reflect a predominant suppression of paradoxical phase of sleep over 4 days. Throughout the study, the experimental room was maintained under controlled temperature ( $22 \pm 1^\circ\text{C}$ ) and a 12-h light-dark cycle (lights on at 7:00 and off at 19:00 h). Food and water were provided *ad libitum* by placing chow pellets and water bottles on a grid located on the top of the tank. The water in the tank was changed daily throughout the PSD period. The duration of 96 h of PSD was chosen since it has been shown that most genital reflexes are produced during this time span (11).

### Experimental protocols

Immediately after the end of the 96-h period, the animals (PSD and controls, N = 8-10/group) were injected with saline or sil-

denafil (0.08; 0.16, and 0.32 mg/kg, *ip*) dissolved in saline and then placed in a transparent cylindrical cage (32 cm in diameter and 30 cm in height). The numbers of spontaneous penile erections and ejaculations were assessed for 60 min. Penile erection was counted only when the rat displayed its penis and bent down to lick it in full erection. The behavioral observations were carried out between 9:00 and 11:00 h, according to the procedure followed in a previous study by our group (12). Different groups of rats and different cages were used in each series of experiments and cages were cleaned thoroughly after each test.

#### Blood sampling and hormone determination

After the experiment, the animals were sacrificed individually by decapitation with a minimum of disturbance in an adjacent room. Blood was collected into glass tubes and centrifuged at 3500 rpm for 10 min to obtain samples of serum or plasma. The samples were maintained at -20°C until the assays. The intra-assay coefficient variations are given in parentheses. Testosterone concentration (6.7%) was measured by a chemiluminescent immunometric assay (Immulite Automated Analyses-Diagnostic Products Corporation, Los Angeles, CA, USA), with a detection limit of 10 ng/dL. Progesterone (6.5%) was measured by competitive immunoassay (TOSOH Corporation, Tokyo, Japan) and the minimal detectable concentration was 0.1 ng/mL. Plasma dopamine (DA) concentrations were determined by ion-pairing reverse phase liquid chromatography coupled with electrochemical detection (15). High-performance liquid chromatography was performed with an instrument (Shimadzu Corporation, Kyoto, Japan) consisting of one LC-10AD VP pump, and one L-ECE-6A electrochemical detector. The detection limit obtained was 40 to 50 pg/mL. All analyses were performed in accordance with our previous study (16) and in duplicate.

#### Statistical analysis

Statistical analysis of the percentages of rats displaying penile erection and ejaculation was analyzed by the two-tailed Fisher test to assess differences between groups. Hormonal data were subjected to analysis of variance (two-way ANOVA test using dose (saline vs sildenafil) and deprivation (control vs PSD) as factors), followed by the Duncan test for comparison between groups. Data are reported as mean  $\pm$  SEM. The level of significance was set at  $P < 0.05$ .

## Results

#### Experiment 1. Effect of sildenafil on genital reflexes

Figure 1 shows the results of the statistical analysis. Five of 10 PSD-saline rats displayed penile erections and 2 of 10 ejaculated. This proportion of penile erection differed from that of home cage rats injected with saline ( $P < 0.03$ ). A single injection of sildenafil at doses ranging from 0.08 to 0.32 mg/kg did not significantly alter the percentage of rats displaying penile erection compared to PSD-saline or to control groups (Fisher test).

The effects promoted by the lower sildenafil doses (0.08 and 0.16 mg/kg) injected into the PSD rats did not differ from those of the control group ( $P > 0.08$ ). An acute sildenafil injection at the dose of 0.32 mg/kg in PSD rats led to only two in 10 rats displaying penile erection, a result that was not statistically significant. No genital reflexes were observed in control rats.

#### Experiment 2. Effect of PSD and sildenafil on hormone concentrations

The testosterone values are presented in Figure 2A. Statistical evaluation by two-way ANOVA revealed only significant effects of dose ( $F_{3,53} = 2.93$ ,  $P < 0.04$ ) but not

Figure 1. Effect of acute sildenafil administration to paradoxical sleep-deprived (PSD) or home cage-maintained (control) rats on genital reflexes (penile erection, PE, and ejaculation, EJ). The dose of sildenafil is given below the columns in mg/kg. \* $P < 0.05$  compared to the control group (ANOVA).

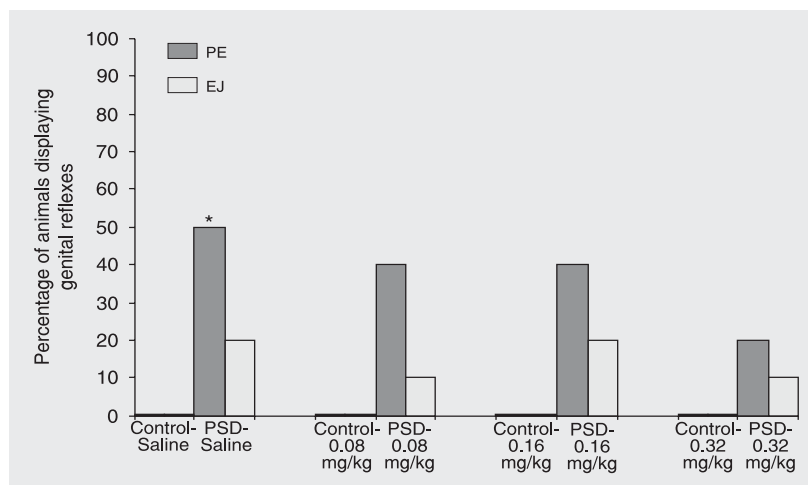
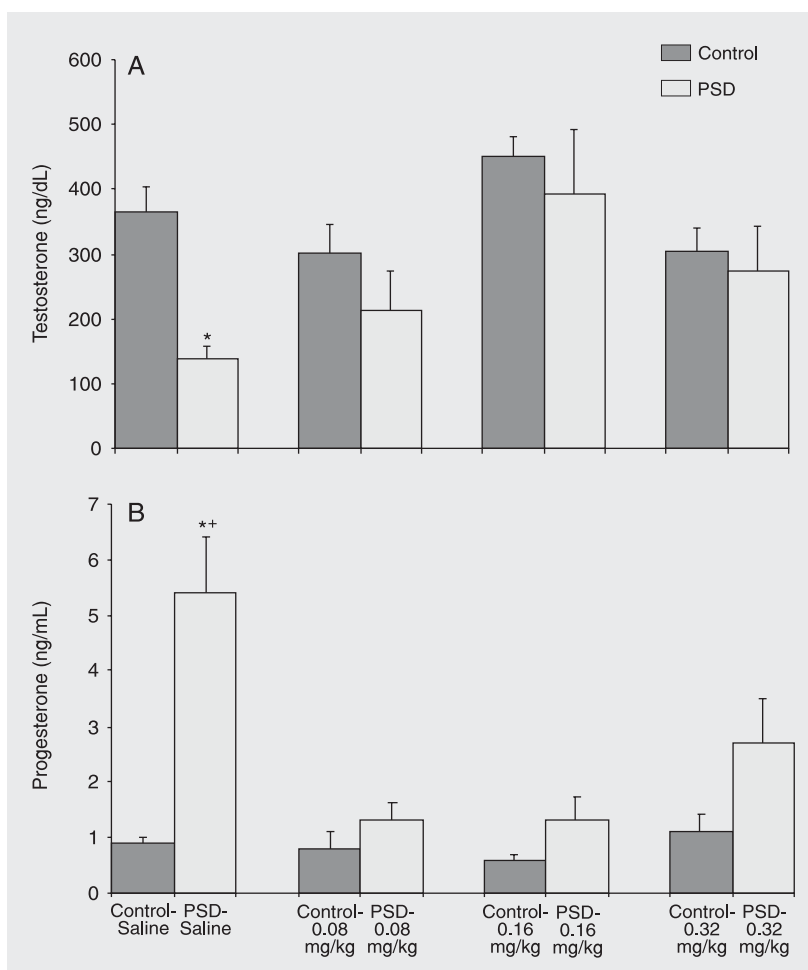


Figure 2. Serum testosterone (ng/dL, A) and progesterone (ng/mL, B) concentrations in control (control) and paradoxical sleep-deprived (PSD) animals after saline or sildenafil injection. The dose of sildenafil is given below the columns in mg/kg. Data are reported as means  $\pm$  SEM. \* $P < 0.05$  compared to the control group; \*\* $P < 0.05$  compared to the PSD-sildenafil groups (ANOVA).



of deprivation or interaction ( $P > 0.05$ ). The *a posteriori* test indicated that PSD significantly reduced the testosterone values in saline-injected rats compared to the respective controls ( $P < 0.03$ ). However, all doses of sildenafil prevented the reduction of testosterone concentrations observed after PSD compared to the respective control group.

Two-way ANOVA revealed significant effects of dose ( $F_{3,53} = 4.09$ ,  $P < 0.01$ ) and deprivation ( $F_{1,53} = 10.77$ ,  $P < 0.001$ ). The *post hoc* Duncan test revealed that the PSD-saline group differed from the respective control group ( $P < 0.001$ ) and from the other PSD-sildenafil-treated rats ( $P < 0.01$ ). There was no significant difference between PSD rats and their respective control groups for each dose of sildenafil. The progesterone values are shown in Figure 2B.

Figure 3 depicts the plasma DA concentrations of control and PSD rats pretreated with saline and different sildenafil doses. Two-way ANOVA revealed significant effects of dose ( $F_{3,53} = 3.05$ ,  $P < 0.03$ ) and deprivation ( $F_{1,53} = 6.93$ ,  $P < 0.01$ ). The *post hoc* Duncan test revealed that the PSD-saline group exhibited higher concentrations of DA compared to its control-saline group ( $P < 0.001$ ). Statistical analysis revealed that sildenafil did not produce a significant effect at any of the three doses tested.

## Discussion

In the present study, we report the effects of PSD as an isolated factor on the occurrence of genital reflexes in male rats and corroborate previously findings showing that 50% of sleep-deprived rats displayed penile erection and 20% of them ejaculated (17). Nevertheless, sildenafil at the doses used in the present study led to loss of statistical significance in the frequency of penile erection, and antagonized the hormonal (18) and plasma DA changes induced by PSD. The effects of sildenafil were antagonistic to those of PSD and appeared to be responsible for

the loss of significance in penile erection frequency.

Ferrari et al. (19) reported that penile erection occurred in sexually experienced (SE) rats injected with 1 mg/kg sildenafil even in the absence of a female, while no significant effect was observed in sexually inexperienced males, as used in our protocol design. However, it should be noted that SE rats had undergone prior mating tests. A plausible explanation for these findings may be the dose used and also the experimental environment, i.e., the observation cages where SE rats had previously performed several mating tests. It has been reported that the specific conditions under which an animal is exposed to a drug and/or natural incentive contribute in the development of "sensitization" that results in a subsequent greater response (20). In men, in the absence of sexual stimulation, sildenafil at the recommended doses has no effect on sexual desire or libido (21). In our model, sexually inexperienced males were placed individually in cages in the absence of sexual stimuli. Presumably, it could be expected that PSD-induced penile erection does not override the effect of sildenafil in the absence of

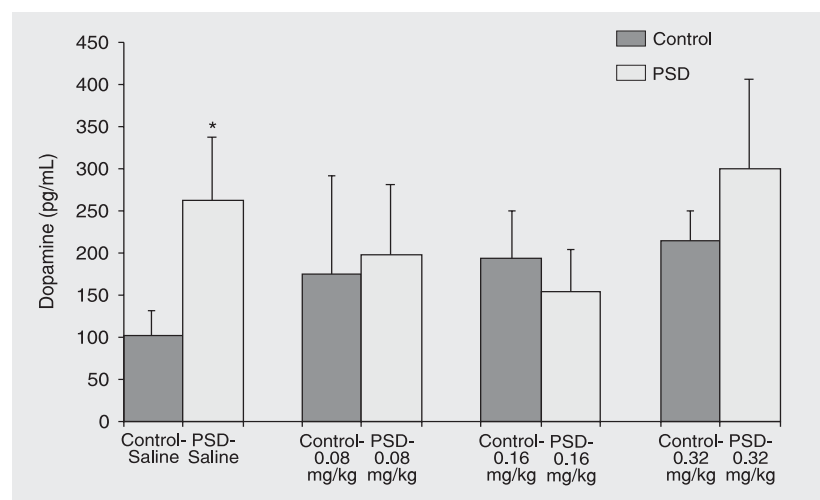


Figure 3. Concentrations of plasma dopamine (pg/mL) of control and paradoxical sleep-deprived (PSD) animals after saline or sildenafil injection. The dose of sildenafil is given below the columns in mg/kg. Data are reported as means  $\pm$  SEM. \* $P < 0.05$  compared to the control group (ANOVA).

sexual stimuli triggered by the presence of a female. Under the conditions of our experimental design, genital reflexes remained unchanged after sildenafil treatment in comparison to the PSD-saline group, indicating that the stimulation of the genital reflexes by sildenafil did not result in potentiating effects in PSD animals. The statistical difference between PSD and control rats, both injected with sildenafil, did not represent a drug response but rather a sleep deprivation-induced effect, since the same frequencies that occurred in the untreated and sildenafil-treated PSD groups were observed.

Regarding the hormonal evaluation of testosterone and progesterone, two steroid hormones involved in the regulation of sexual behavior, acute sildenafil administration did not result in any statistical differences between control and PSD groups, whereas sleep deprivation was sufficient to induce a marked alteration (22) in these hormones, as shown in our previous studies (12,16,18). Indeed, sildenafil prevented not only the reduction of testosterone but also the increase of progesterone concentrations observed in PSD rats. In fact, the effects of testosterone on male sexual motivational have been recognized (23), whereas the physiological role of progesterone in male sexual behavior is less clearly understood (18). In this context, sleep-deprived rats showed significantly lower testosterone concentrations, indicating that testosterone *per se* cannot be considered to be the main factor governing the genital behaviors observed in the PSD groups, as previously reported. This also indicates that the action of sildenafil is dependent on testosterone, as pointed out by Ottani et al. (24). Thus, the normalization of testosterone concentrations induced by sildenafil may be considered to be incompatible with the penile erection induced by PSD. These data lead to the interpretation that penile erection as a consequence of PSD is governed by an inducing mechanism other than that of sildenafil, i.e., the first would be testosterone

independent (as observed in prior investigations by our group and leading to the hypothesis concerning the relevance of progesterone), while the mechanism governing the effects of sildenafil would be testosterone dependent. Since in PSD subjects penile erection occurs at low testosterone concentrations, whose normalization by sildenafil causes the frequency of penile erection lose its statistical significance, it can be deduced that testosterone reduction in PSD is a permissive factor that bears weight on the inducing action of penile erection by yet another factor, most likely progesterone. The choice of using doses lower than those normally described in the literature can be ascribed to the intention of verifying potentiation without the interference of the ceiling effect (the dose of 1 mg/kg sildenafil induces erection in 80% of SE rats) (19). Any increase in this percentage (potentiation) would not be detected by statistical analysis since it would be too close to the 100% limit. Of note, the doses used here seem to have been the right ones since they permitted the confirmation of underlying mechanisms. Nevertheless, one should not ignore the possibility of higher doses of sildenafil unleashing the full intensity of its erection mechanisms and overriding the effects of sleep deprivation, even when these have their own independent mechanisms.

Based on the evidence that DA is the main neurotransmitter of sexual behaviors (25-27), and its release is modulated by NO (28,29), Ferrari et al. (19) reported that pretreatment of SE rats with 1 mg/kg sildenafil potentiated the penile erection induced by the DA agonists, indicating that sildenafil might play a facilitatory role in sexual responses, further augmenting DA stimulation provoked by the expectation of copulatory behavior in SE rats (30). In addition, sildenafil increased and prolonged apomorphine-evoked intracavernous pressure in the rat, thus suggesting a possible combination of the two drugs for the management of erectile

dysfunctions (31). As can be seen in Figure 3, plasma DA levels did not differ between control and sleep-deprived subjects in the sildenafil-treated groups. The only significant effect detected was between the control and PSD-saline groups, which led to an increase of DA concentrations in PSD animals ( $103.4 \pm 30.7$  vs  $262.6 \pm 77.7$  pg/mL), as also shown in our previous studies.

Concerning the magnitude of the effects, the PSD method seems to exert a favorable effect on male genital reflexes. Together with our previous data in this series of experiments (9-12), the present observations support the notion that this sexual behavioral effect is a consequence of the changes in the levels of several brain monoamines and subsequent physiological alterations induced by sleep deprivation. Although additional factors must be involved in such a complex phenomenon like sexual behavior, this study adds evidence about some factors involved in genital reflexes induced by PSD in males. Testosterone, progesterone and DA may rep-

resent relevant factors that exert influence on genital reflexes in PSD males.

Our results suggest that, although sildenafil did not potentiate erection or ejaculation in PSD rats, the stimulating effects of PSD associated with cocaine on erection can be modified by alterations in the NO system (32). Moreover, sildenafil prevented the hormonal alterations induced by sleep deprivation. Additional studies are underway in our laboratory to elucidate the role of NO and DA in genital reflexes displayed by sleep-deprived rats.

### Acknowledgments

The authors would like to express their thanks to Marilde Costa, Waldemarks Leite, and Tomé Pimentel for assistance during the project, Dulce Elena Casarini and Luciana Teixeira of the Department of Medicine, Nephrology section, for assistance with HPLC, and Angela Reksidler and Marcelo Lima for valuable comments.

### References

1. Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochem Biophys Res Commun* 1990; 170: 843-850.
2. Azadzi KM, Kim N, Brown ML, Goldstein I, Cohen RA, Saenz de Tejada I. Endothelium-derived nitric oxide and cyclooxygenase products modulate corpus cavernosum smooth muscle tone. *J Urol* 1992; 147: 220-225.
3. Snyder SH. Nitric oxide: first in a new class of neurotransmitters. *Science* 1992; 257: 494-496.
4. Marletta MA, Yoon PS, Iyengar R, Leaf CD, Wishnok JS. Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. *Biochemistry* 1988; 27: 8706-8711.
5. Bredt DS, Snyder SH. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proc Natl Acad Sci U S A* 1989; 86: 9030-9033.
6. Reilly CM, Lewis RW, Stopper VS, Mills TM. Androgenic maintenance of the rat erectile response via a non-nitric-oxide-dependent pathway. *J Androl* 1997; 18: 588-594.
7. Boolell M, Allen MJ, Ballard SA, Gepi-Attee S, Muirhead GJ, Naylor AM, et al. Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. *Int J Impot Res* 1996; 8: 47-52.
8. Nachtsheim D. Sildenafil: a milestone in the treatment of impotence. *West J Med* 1998; 169: 112-113.
9. Andersen ML, Bignotto M, Tufik S. The effect of apomorphine on genital reflexes in male rats deprived of paradoxical sleep. *Physiol Behav* 2003; 80: 211-215.
10. Andersen ML, Tufik S. The effects of dopaminergic agonists on genital reflexes in paradoxical sleep-deprived male rats. *Physiol Behav* 2005; 84: 205-210.
11. Andersen ML, Bignotto M, Tufik S. Cocaine-induced genital reflexes during paradoxical sleep deprivation and recovery. *Physiol Behav* 2003; 78: 255-259.
12. Andersen ML, Papale LA, Tufik S. Diurnal variation in the genital reflexes and hormone levels induced by paradoxical sleep deprivation and cocaine in male rats. *Brain Res Bull* 2004; 64: 215-220.
13. Giuliano F, Allard J. Dopamine and sexual function. *Int J Impot Res* 2001; 13 (Suppl 3): S18-S28.
14. Chan JY, Huang CL, Chan SH. Nitric oxide as a mediator of cocaine-induced penile erection in the rat. *Br J Pharmacol* 1996; 118: 155-161.
15. Naffah-Mazzacoratti MG, Casarini DE, Fernandes MJS, Cavalheiro EA. Serum catecholamine levels determined by high performance liquid chromatography coupled with electrochemical detection. *Arq Bras Endocrinol Metabol* 1992; 36: 119-122.

16. Andersen ML, Martins PJ, D'Almeida V, Bignotto M, Tufik S. Endocrinological and catecholaminergic alterations during sleep deprivation and recovery in male rats. *J Sleep Res* 2005; 14: 83-90.
17. Andersen ML, Tufik S. Distinct effects of paradoxical sleep deprivation and cocaine administration on sexual behavior in male rats. *Addict Biol* 2002; 7: 251-253.
18. Andersen ML, Tufik S. Does male sexual behavior require progesterone? *Brain Res Rev* 2006; 51: 136-143.
19. Ferrari F, Ottani A, Giuliani D. Influence of sildenafil on central dopamine-mediated behaviour in male rats. *Life Sci* 2002; 70: 1501-1508.
20. Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol* 1993; 4: 289-312.
21. Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. *N Engl J Med* 1998; 338: 1397-1404.
22. Tufik S, Lindsey CJ, Carlini EA. Does REM sleep deprivation induce a supersensitivity of dopaminergic receptors in the rat brain? *Pharmacology* 1978; 16: 98-105.
23. Roselli CE, Chambers K. Sex differences in male-typical copulatory behaviors in response to androgen and estrogen treatment in rats. *Neuroendocrinology* 1999; 69: 290-298.
24. 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: 717-722.
25. Bitran D, Hull EM. Pharmacological analysis of male rat sexual behavior. *Neurosci Biobehav Rev* 1987; 11: 365-389.
26. Melis MR, Argiolas A. Dopamine and sexual behavior. *Neurosci Biobehav Rev* 1995; 19: 19-38.
27. Ferrari F, Giuliani D. Sexual attraction and copulation in male rats: effects of the dopamine agonist SND 919. *Pharmacol Biochem Behav* 1995; 50: 29-34.
28. Melis MR, Succu S, Argiolas A. Dopamine agonists increase nitric oxide production in the paraventricular nucleus of the hypothalamus: correlation with penile erection and yawning. *Eur J Neurosci* 1996; 8: 2056-2063.
29. Hull EM, Lorrain DS, Du J, Matuszewich L, Lumley LA, Putnam SK, et al. Hormone-neurotransmitter interactions in the control of sexual behavior. *Behav Brain Res* 1999; 105: 105-116.
30. Mas M. Neurobiological correlates of masculine sexual behavior. *Neurosci Biobehav Rev* 1995; 19: 261-277.
31. Andersson KE, Gemalmaz H, Waldeck K, Chapman TN, Tuttle JB, Steers WD. The effect of sildenafil on apomorphine-evoked increases in intracavernous pressure in the awake rat. *J Urol* 1999; 161: 1707-1712.
32. Andersen ML, Perry JC, Antunes IB, Tufik S. Involvement of nitric oxide in cocaine-induced erections and ejaculations after paradoxical sleep deprivation. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 31: 652-657.