Impairment of male reproductive function after sleep deprivation

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Objective: To evaluate the influence of sleep loss on sexual behavior, hormone levels, sperm parameters, and testis-specific gene expression in male rats.
Design: Experimental research.
Setting: Animal laboratory.
Animal(s): Male adult Wistar-Hannover rats.
Intervention(s): Sexually experienced rats were subjected to paradoxic sleep deprivation (PSD) for 96 hours or sleep restriction (SR) for 21 days or kept in their home cage as control (CTRL).
Main Outcome Measure(s): Sexual behavior, hormone levels, sperm parameters and expression of stress and nitric oxide–related genes were evaluated.
Result(s): PSD significantly decreased sexual behavior compared with the CTRL group, whereas SR had no effect. The PSD group had significantly lower testosterone levels than the CTRL group. Both PSD and SR groups had lower sperm viabilities than the CTRL group. The decrease in the number of live sperm compared with the CTRL group was larger in the PSD group than in the SR group. Regarding testicular gene expression, both PSD and SR led to an increase of iNOS and hydroxysteroid 11β-dehydrogenase 1 expressions compared with the CTRL group. These changes were more pronounced in the PSD group. A significant increase in endothelial nitric oxide synthase expression was observed in the PSD groups compared with the CTRL group. No changes were observed in dimethylarginine dimethylaminohydrolase 1 and casein kinase 2β-polypeptide expressions.
Conclusion(s): Sleep loss can promote marked changes in the male reproductive system of rats, particularly affecting spermatic function in part by interfering in the testicular nitric oxide pathway. (Fertil Steril® 2015;103: 1355–62. ©2015 by American Society for Reproductive Medicine.)
Key Words: Sleep restriction, sexual behavior, testosterone, progesterone, sperm, reproduction, nitric oxide, male rat, iNOS, eNOS

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Reproductive function in humans has been of particular concern in recent years. Diseases, psychologic factors, stress, and hormonal changes are some of the factors that contribute to the appearance of dysfunction in the male reproductive system (1). Studies have shown that sperm concentration has been decreasing over the years (2–5) and a high prevalence of erectile dysfunction complaints has been observed in men 18–40 year old in association with psychosocial but not organic problems (6). The stress resulting from socioeconomic pressure combined with increased workload lead to a decrease of total sleep time. Because it is difficult to study the isolated role of sleep loss in human reproduction, it becomes valuable to use nonhuman models.

In rats, adverse effects of sleep deprivation (SD) have been well documented and include changes in functional parameters of male sexual behavior, leading to increased frequency of spontaneous erections and ejaculations (7, 8). To comprehensively assess sexual behavior, one must scrutinize other motivational behaviors, expressed as performance, such as the numbers of mounts, intromissions, and ejaculations. Alvarenga et al. (9) demonstrated that rats exposed to 96 hours of paradoxic SD (PSD) displayed reduced sexual performance, as evidenced by an increased latency for intromission initiation and a reduction in the

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number of intromissions compared with a control group. In addition to altering sexual behavior, PSD has been found to influence sex hormones. Male rats exhibit marked hormonal alterations when subjected to PSD, with decreases in testosterone (T) and E_2 concentrations, as well as increases in progesterone (P) and glucocorticoid levels (10–12).

Progesterone, LH, FSH, and T are all intimately involved in the process of cellular division and spermatogenesis, which ultimately leads to spermatozoid production (13–17). Some specific genes are also responsible for spermatogenesis and infertility (18, 19). In addition to the important role of nitric oxide (NO) in male fertility, mainly on the effectiveness of erection for sexual intercourse, NO can be toxic to cells at high levels, owing to the inhibition of DNA replication and lipid peroxidation (20). Inducible nitric oxide synthase (iNOS), a calcium-independent NOS present in the testis, is implicated in spermatogenesis and apoptosis of Sertoli and germ cells (21). Therefore, we hypothesized that both acute and chronic SD may lead to endocrine alterations, adversely affecting sexual behavior and sperm quality and quantity in association with molecular modulation of the NO pathway in the reproductive system of male rats.

MATERIALS AND METHODS

Animals

Adult male Wistar-Hannover rats were bred and raised in the animal facility of the Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia of the Universidade Federal de São Paulo. The animals were housed in a colony with a constant temperature of 22 ± 1°C and a 12-h/12-h light/dark cycle (lights on at 07:00) and had free access to water and food. All animals were treated in accordance with the National Institutes of Health guidelines, and all procedures were approved by the university's Ethics Committee (CEP no. 09/071).

Training and Sexual Behavior Evaluation

Before sexual behavior was evaluated, the rats acquired sexual experience through training. Because sexually inexperienced male rats can display low performance, we followed an established protocol that standardizes the degree of copulatory activity and avoids possible bias (22). Twenty-four hours after the last training session, the rats with excellent sexual performance (i.e., animals that showed >70% ejaculation frequency during the training) were selected and subjected to PSD for 96 hours or sleep restriction (SR) for 21 days. After these periods, sexual behavior was reevaluated immediately.

Training and testing of sexual behavior was performed with the use of a Plexiglas cylinder arena with a 45-cm diameter. Dim red lights shone during the dark phase of the light/dark cycle. A male rat was introduced into the arena 5 minutes before a female rat. Sexual receptivity in the female rats was established by subcutaneous administration of E_2 benzoate (10 μg/0.1 mL sesame oil; Sigma Chemical Co.) 48 hours and 24 hours before testing, followed by subcutaneous administration of P (500 μg/0.1 mL sesame oil; Sigma Chemical Co.) 4 hours before testing sexual behavior. Each test of sexual behavior lasted for 30 minutes after the introduction of the female rat, during which the following variables were recorded: time to first mount; intromission and ejaculation latencies; total numbers of mounts (i.e., mounts with pelvic thrusting); intromissions (mounts with pelvic thrusting and penile insertion); and ejaculations. The copulation rate (number of intromissions/[number of mounts + number of intromissions]), inter-intromission interval (ejaculation latency/number of intromissions) and intercopulatory interval (ejaculation latency/[number of intromissions + number of mounts]) were also calculated (23).

Protocol Designs

The animals that displayed excellent performance after sexual training were randomly assigned to one of the following three groups (n = 10 per group): CTRL, control rats maintained in their home cage; s PSD, rats submitted to 96 hours of PSD; and SR, rats submitted to 21 days of SR.

Paradoxic Sleep Deprivation

Rats were subjected to 96 hours of PSD by means of the modified multiple-platform method. The 96-hour length of the PSD was chosen based on previous studies showing that the most dramatic alterations in behavior (24) and hormone concentrations (11) occur for this period of PSD. The ten rats were individually placed inside a tiled water tank (143 × 41 × 30 cm) containing 14 circular platforms (each 6.5 cm in diameter) with the water level within 1 cm of the upper surface. The rats could move within the tank by jumping from one platform to another. When they reached the paradoxic phase of sleep, muscle atonia caused them to fall into the water and awaken. Throughout the study, the experimental room was maintained at a controlled temperature (22 ± 1°C) with a 12-h/12-h light/dark cycle (lights on from 07:00 to 19:00). The rats had free access to food and water located on a grid at the top of the tank. The water in the tank was changed daily during the PSD period. All animals began their PSD period at the same time in the dark phase of the light/dark cycle (19:00). Because we elected not to invert the light/dark cycle, the rats were trained and tested during a dark phase.

Sleep Restriction

The SR protocol was based on the technique used for the PSD conditions. The difference in the SR protocol was that the rats were kept on the platforms for 18 hours (beginning at 16:00) and allowed to sleep for 6 hours (10:00–16:00) every day for 21 days, providing partial compensation for sleep loss (24). The time interval of 10:00–16:00 was chosen because it represented the time when paradoxic sleep is at its highest (25).

Sperm Evaluation

Immediately after male ejaculation, the female rat was killed and the seminal fluid directly removed from the uterine horns. Seminal fluid was stored in Eppendorf tubes at 37°C and subjected to microscopic and macroscopic analyses (26).

For examination of sperm viability, seminal fluid (10 μL) was placed on a slide with eosin-nigrosine dye (10 μL), which allowed us to count the number of live (nonstained) and dead (stained) sperm. To prepare the dye, eosin (1 g) was diluted in distilled water (100 mL), followed by the addition of nigrosine (5 g) and sodium citrate (2.9 g). The dye was filtered and stored in amber bottles.

For measurement of sperm concentration, we carried out a 1:100 dilution of the seminal fluid (10 μL) with the use of 10% formalin (90 μL) and counted the sperm with the aid of a Neubauer chamber. For evaluation of sperm mobility, 100 sperm were classified according to the types of movement as follows: fast progressive; slow progressive; in situ; or quiet (no movement). For assessment of morphology, 100 sperm were classified as follows: normal; only a tail; only a head; or two heads. The following macroscopic variables of the seminal fluid were examined: color (yellow, white or transparent); volume; and pH level.

Blood Sampling and Hormone Determination

Immediately after behavioral testing, all of the rats in the CTRL, PSD, and SR groups were taken to an adjacent room and decapitated. Blood samples were collected and stored individually. Blood was collected in glass tubes, centrifuged at 3,018.4 *g* for 15 minutes at room temperature and frozen at −20°C until analysis. The serum T (intra-assay coefficient of variation [ICV] 7.7%) and P (ICV = 6.5%) levels were measured with the use of a chemiluminescent enzyme immunoassay (Advia Centaur; Bayer Corporation). LH and FSH (ICV 9.9%) were measured with the use of Multiplex/Luminex technology (Millipore).

Tissue Collection and Total RNA Extraction

The animals were decapitated immediately after the sexual behavior test. The right testis was rapidly dissected, flash-frozen on dry ice, and then stored at −80°C until RNA extraction. Total RNA was extracted from the testis with the use of Trizol reagent (Invitrogen) according to the instructions of the manufacturer. After extraction, RNA was evaluated by means of electrophoresis in agarose gel to assure integrity of the 18S and 28S ribosomal subunits.

Reverse Transcription and Quantitative Real-Time Polymerase Chain Reaction

Total RNA (2 μg) was reverse transcribed into cDNA with the use of the High-Capacity cDNA Reverse Transcription Kit (Life Technologies) according to the instructions of the manufacturer. Reverse transcription was performed at 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes. Amplification (400 ng cDNA) and detection was performed with the use of Assay-on-Demand and Taqman 5 fluorogenic nuclease chemistry in an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems) according to the instructions of manufacturer. A two-stage cycle (hold stage of 50°C for 2 minutes and 95°C for 10 minutes, followed by 95°C for 15 seconds and 60°C for 1 minute) was repeated 40 times and followed by a dissociation stage. Identifications of primers for rat iNOS2, rat endothelial nitric oxide synthase (eNOS), rat dimethylarginine dimethylaminohydrolase 1 (Ddah1), hydroxysteroid 11β-dehydrogenase 1 (Hsd11b1), and casein kinase 2β-polypeptide (Csnk2b) target genes were Rn00561646_m1, Rn02132634_s1, Rn00574200_m1, Rn00567167_m1, and Rn01525929_m1, respectively. Endogenous control genes chosen for normalization were β-actin (reference sequence NM_031144) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; reference sequence NM_017008), whose primer sequences (5′–3′) are, respectively, AGCGTGGCTACAGCTTCACC/AAGTCTAGGGCAACATAGCACAGC and TGCCCCCATGTTTGTGATG/GCTGACAATCTTGAGGGAGTTGT.

Statistical Analysis

Sexual behavior data were assessed for normality and homogeneity with the use of the Shapiro-Wilk and Levene tests, respectively. To meet the necessary prerequisites for the use of parametric tests, it was necessary to standardize the data with the use of z-score. Subsequently, we used one-way analysis of variance (ANOVA) followed by a Tukey post hoc test when required. The hormonal and semen data were analyzed by means of one-way ANOVA followed by a Tukey post hoc test to compare individual group pairs.

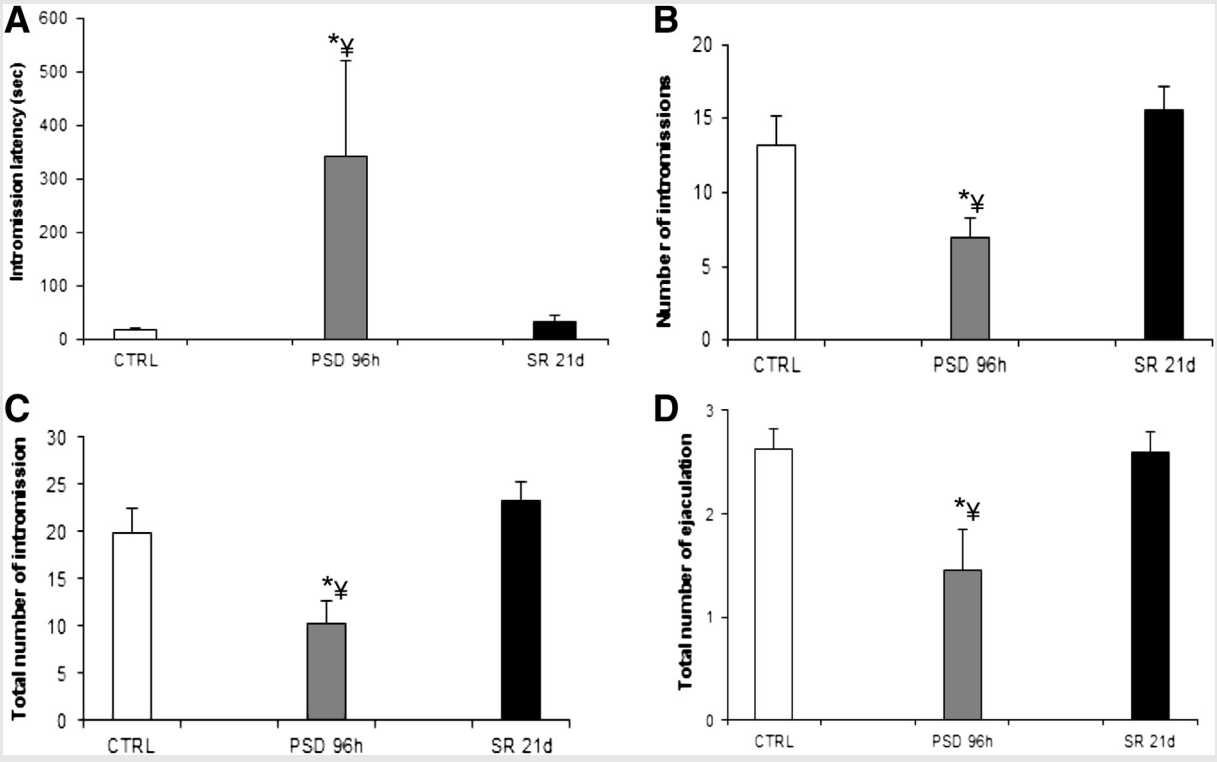
The expression fold changes of all genes were calculated by means of the $2^{-\Delta\Delta Ct}$ method (27) using the arithmetic mean of the reference genes, GAPDH and β-actin, as the normalization factor because they did not differ between groups. The variables were first tested for normality and homogeneity distribution, and all of them fit the parametric criteria, except the iNOS-relative gene expression values. Therefore, for that variable we used a rank-based nonparametric analysis (Kruskal-Wallis) followed by Games-Howell test, which is a post hoc test suitable for unequal variance. We used the Monte Carlo method for computing the significance level and considered 99% to be the confidence interval. For eNOS, Hsd11b1, Ddah1, and Csnk2b gene expression, ANOVA was applied, followed by Tukey post hoc tests. The analyses were performed with the use of SPSS software (version 17). Data are expressed as mean ± SEM. The level of significance was $P<.05$.

RESULTS

Sexual Behavior Parameters

Significant differences were found in the intromission parameters. The PSD group had a longer latency to first intromission compared with the CTRL and SR groups ($F_{2,23} = 9.42$; $P<.001$ [$P<.002$ and $P<.003$, respectively]; Fig. 1A). The PSD group displayed a significant decrease in the number and total number of intromissions ($F_{2,21} = 3.87$; $P<.008$; and $F_{2,24} = 5.20$; $P<.002$ [$P<.03$ and $P<.009$, respectively; Fig. 1B; and $P<.03$ and $P<.002$, respectively; Fig. 1C]) and ejaculations ($F_{2,24} = 6.43$; $P<.001$ [$P<.001$]; Fig. 1D) compared with the CTRL and SR groups. The SR group showed no significant differences from the CTRL group for any of the measures of sexual behavior, including the parameters of latency and number of mounts (Fig. 1).

FIGURE 1



Effects of paradoxic sleep deprivation (PSD) and sleep restriction (SR) on (**A**) latency to intromission, (**B**) number of intromissions, and total numbers of (**C**) intromissions and (**D**) ejaculations. The data are expressed as mean ± SEM. (**A**, **B**) *$P<.05$: significant difference versus the control (CTRL) group; ¥$P<.05$: significant difference versus the SR group. (**C**, **D**) *$P<.03$: significant difference versus the CTRL group; ¥$P<.002$: significant difference versus the SR group.

Alvarenga. Sleep loss affects rat sperm function. Fertil Steril 2015.

The PSD group showed significant increases in the intercopulatory interval ($P<.01$), interintromission interval ($P<.01$) and copulatory rate ($P<.01$) compared with the CTRL and SR groups (Table 1). The SR group showed a similar rate and similar intervals compared to the CTRL group (Table 1).

Hormone Concentrations

ANOVA followed by a Tukey test revealed a significant decrease (45%) in the T concentration in the PSD group compared with the CTRL group ($P<.01$). Figure 2B shows the effects of PSD and SR on the P levels. ANOVA revealed no significant differences among the three groups ($P>.05$). In addition, FSH and LH concentrations showed no significant changes in the PSD and SR groups compared with the CTRL group ($P>.05$).

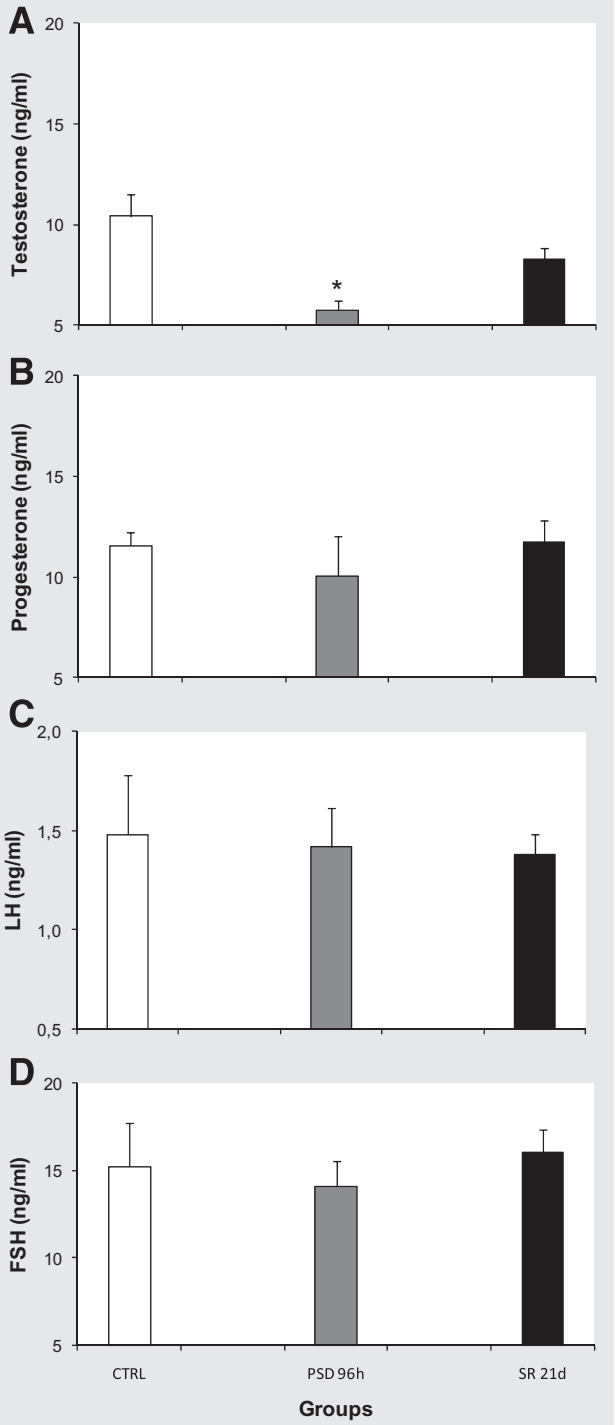
Testicular Gene Expression

ANOVA revealed a significant group effect ($F_{2,26} = 8.09$; $P<.01$) on eNOS gene expression, demonstrating that PSD rats (0.67 ± 0.05) had decreased gene expression in the testis compared with CTRL (1.03 ± 0.08; $P<.01$) and SR (0.96 ± 0.07; $P<.05$) rats (Fig. 3A). Regarding relative iNOS gene expression in the testis from the different groups (Fig. 3B), Kruskal-Wallis analysis showed significant differences between groups ($\chi^2 = 20.43$; $P<.0001$), and Games-Howell post hoc test revealed that both PSD (rank = 22.20; $P<.001$) and SR (rank = 15.88; $P<.01$) led to increases in iNOS expression compared with CTRL. Also, PSD differed from SR ($P<.05$). Regarding the gene expression of the Ddah1 enzyme in the testis (Fig. 3C), ANOVA revealed no group effect ($F_{2,24} = 0.28$; $P>.05$). Regarding Hsd11b1 expression levels (Fig. 3D), statistical analysis showed a group effect ($F_{2,23} = 6.07$; $P<.01$) with a significant increase in Hsd11b1 gene expression in PSD (1.48 ± 0.05) and SR (1.44 ± 0.18) groups compared with CTRL (1.00 ± 0.08). No changes were observed in Csnk2b mRNA levels (Fig. 3E) between the groups ($F_{2,26} = 2.32$; $P>.05$).

Semen Analysis

Microscopic variables of sperm concentration, viability, and mobility. No significant differences in the sperm concentrations were observed among the CTRL, PSD, and SR groups. However, both PSD and SR groups showed significantly fewer spermatozoa with faster movement compared with the CTRL group ($P<.01$; Supplemental Table 1). Importantly, the sperm viability was decreased by 50% in the PSD group compared with the CTRL group ($P<.01$). The SR group also had significantly lower sperm viability (15%) compared with the CTRL group ($P<.01$; Supplemental Table 1).

FIGURE 2



Concentrations of serum (**A**) testosterone and (**B**) progesterone and plasma (**C**) LH and (**D**) FSH in the CTRL, PSD, and SR groups. The data are expressed as mean ± SEM. *$P<.01$: significant difference versus the CTRL group. Abbreviations as in Figure 1.

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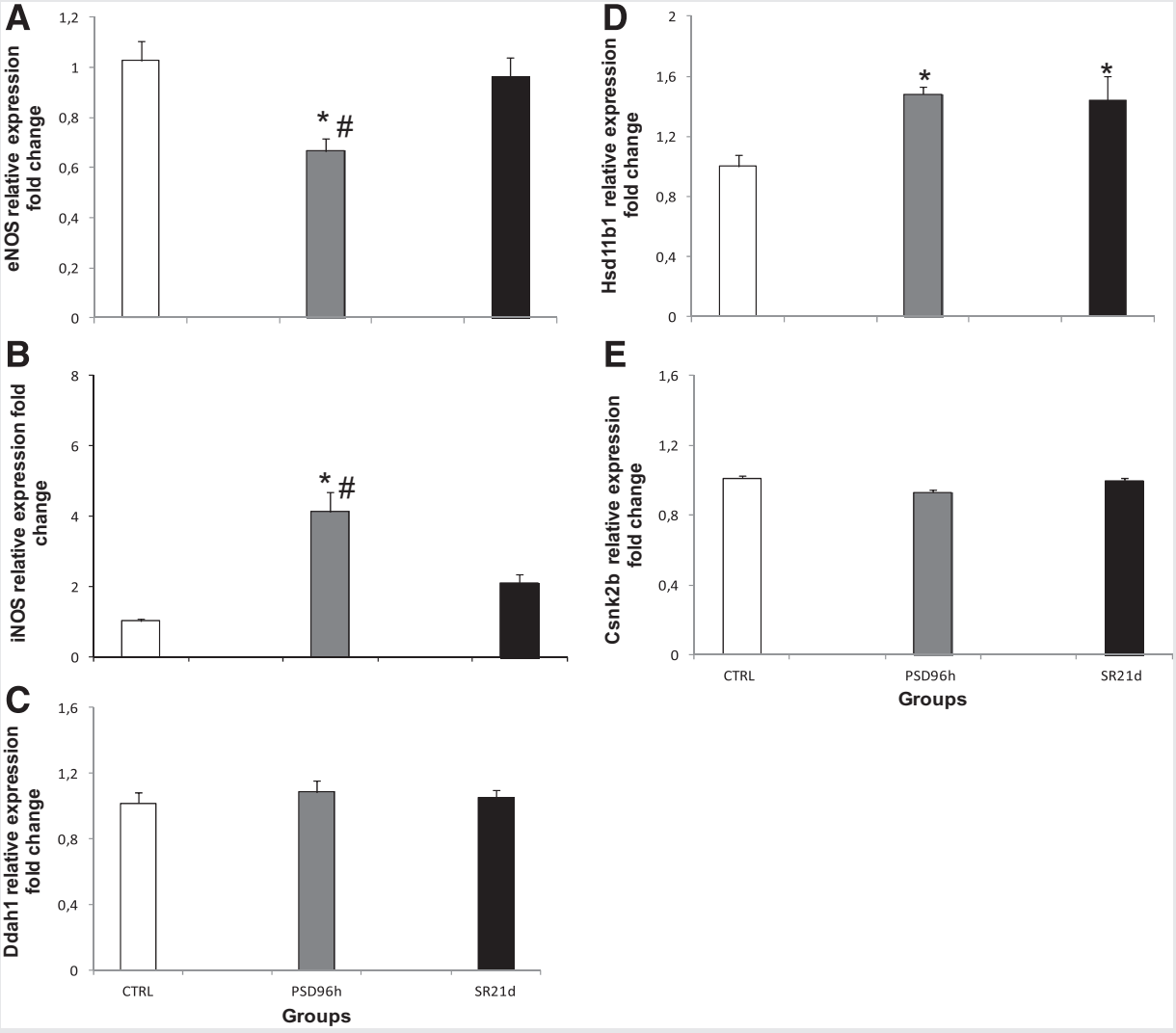
Macroscopic parameters of semen volume and pH. No significant differences were observed in the volume or pH of the seminal fluid among the CTRL, PSD, and SR groups (Supplemental Table 1). Notably, the semen volume was decreased ~50% in the PSD group compared with the CTRL and SR groups, but the differences did not reach statistical significance (Supplemental Table 1).

DISCUSSION

In this study, 96 hours of PSD hampered sexual behavior in male rats that previously had excellent sexual behavior. This effect was reflected in an increased latency to initiate intromission behavior and reduced numbers of intromissions and ejaculations, which previously have been shown to represent lower sexual performance (9). However, when the sleep period was shortened for a longer duration in an attempt to mimic the chronic sleep debt present in human lifestyle, we found no significant alterations in motivation or sexual performance. It seemed that the SR was more subtle and did not alter sexual response of these animals. These responses may have been influenced by the actions of sex hormones. Previously, it was demonstrated that rats submitted to PSD for 96 hours have decreased T concentration (9–11, 28). Testosterone supplementation is an efficient means to maintain and even improve sexual response in adults and elderly male rats (29–31). There is evidence that supplementation of T combined with E_2 shows better results (32). It is plausible to speculate that T replacement combined or not with E_2 or P during the period of SD could improve sexual performance of these animals. Our results suggest that sleep amount modulates T levels, which in turn may contribute, at least in part, with the changes in sexual performance and sperm parameters.

In the present study, we did not find significant differences in the absolute number of sperm. However, other parameters, such as motility and viability, may also be indicative of infertility. Singla and Challana (33) demonstrated that decreased sperm viability resulted in a 100% reduction of pregnancy rate in female rats. Our findings indicate that sleep loss can impair the spermatic viability, and we suppose that these effects may be caused by disruption of the spermatic cycle maintenance (15, 16) owing to a decline in T (9–11, 28). The relationships between endogenous T and sex, sleep, and sleep disturbances have been debated (34), including results of clinical trials as well as nonhuman animal studies. However, not only T may have mediated the sperm viability reduction, because only the PSD group showed an impairment of sexual performance and T levels, whereas both PSD and SR led to negative changes in the sperm quality and quantity. A recent study demonstrated that decrease in total T, but not in bioavailable T, was related to erectile dysfunction complaints (35). Our findings indicate that, indeed, this hormone plays a role directly proportional to sexual performance. Therefore, we can not definitively exclude the action of T on the sexual and spermatic response of these animals in both sleep-deprived groups. Recently, we published a study showing that the offspring of fathers undergoing PSD or SR presented a decline in the sexual response, accompanied by a reduction in T concentrations (36), revealing far-reaching consequences of sleep loss in reproduction.

FIGURE 3



Expression of **(A)** endothelial nitric oxide synthase (eNOS), **(B)** inducible nitric oxide synthase (iNOS), **(C)** dimethylarginine dimethylaminohydrolase 1 (Ddah1), **(D)** hydroxysteroid 11b-dehydrogenase 1 (Hsd11b1), and **(E)** casein kinase 2b-polypeptide (Csnk2b). **(A)** *$P<.05$ compared with CTRL; #$P<.05$ compared with SR. **(B)** *$P<.01$ compared with CTRL; #$P<.05$ compared with SR. **(C)** No statistical differences. **(D)** *$P<.05$ compared with CTRL. **(E)** No statistical differences. The expression was measured by means of reverse-transcription quantitative polymerase chain reaction (PCR) in the testes of CTRL (non -sleep-deprived control), PSD (paradoxic sleep -deprived), and SR (sleep-restricted) rats.

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The exposure to stressful stimuli seems to alter testicular and plasma T concentrations in a biphasic manner (37, 38). Although there is some evidence that the inhibition of testicular androgen activity observed in chronic stress is associated with reduction in GnRH and LH, this is not always followed by LH reduction (38), as observed in the present study. Therefore, it is possible that corticosterone may be responsible for this feedback block, because the reduction of sleep time is known to increase glucocorticoid levels. However, it is difficult to separate stress from the lack of sleep itself (39). Although PSP led to increased levels of corticosterone, no significant changes were observed in the animals of SR group, suggesting that maybe they have suffered an adaptation by long exposure time in the chronic sleep deprivation. Stress may have dual effect on the HPA axis: adaptation due to repeated exposure or potentiation/facilitation of the response due to a new acute stressor (40). Mazaro and Lamano-Carvalho (41) showed that rats exposed during the neonatal period to maternal deprivation and neonatal stimulation showed reduced basal corticosterone levels compared with a control group, but not when being exposed to a new acute stressor of immobilization. Stress has been shown to interfere in male and female reproductive capability in various animal species (9, 10), and curtailment of sleep has an inherent stress component (11).

In the present study, we also proposed to evaluate some genes that could help us to clarify the mechanisms involved in decreased sperm viability after lack of sleep. We highlight

TABLE 1

Effect of paradoxic sleep deprivation (PSD) or sleep restriction (SR) on intercopulatory interval, interintromission interval, and copulatory rate.

Sexual behavior parameter	Control	PSD	SR
Intercopulatory interval	29.4 ± 2.6	80.6 ± 23.9[a,b]	28.7 ± 4.6
Interintromission interval	41.6 ± 3.7	136.8 ± 35.4[a,b]	48.9 ± 8.3
Copulatory rate	0.6 ± 0.1	0.3 ± 0.1[a,b]	0.6 ± 0.0

[a] Significant difference compared with control group ($P<.01$).
[b] Significant difference compared with SR group ($P<.01$).

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the genetic contribution to testicular function. Some candidate genes were selected, especially those involved with cell apoptosis and NO pathway. Most of the NO produced in the testis is originated from activated testicular macrophages, which have high levels of iNOS (42). Although the positive effects of NO lead to erection, NO can be toxic to cells at high levels, owing to the inhibition of DNA replication and lipid peroxidation (21), and the increase of NO synthesis due to up-regulation of iNOS has been described as responsible for cellular injury and apoptosis (43). Accordingly, the high expression of iNOS and eNOS are related to apoptosis of Sertoli and germ cells in the testis as well as spermatogenesis (18–22). In the present study, an increase of NO generators was observed in both PSD and SR groups, indicating that these animals may show signs of infertility in association with decreased sperm viability and motility (because sperm quantity or concentration was not affected) due at least in part to NO modulation. Corroborating these findings, a significant increase in Hsd11b1 expression levels was also found in the PSD and SR groups, suggesting that the excess of this gene expression may disrupt sperm formation and thus regulation of spermatogenesis (44). On the other hand, the gene expression of testis Ddah1, which acts in the regulation of NO production by inhibition of a NOS endogenous inhibitor (asymmetric dimethyl-L-arginine), was not significantly changed after PSD or SR. The same occurred with Csnk2b expression, which has a regulatory function in cell proliferation, cell differentiation, and apoptosis, being associated with infertility (45). Finally, genes whose expressions are affected in opposite directions by PSD and SR could be potential candidates for understanding sleep homeostasis interaction with male reproduction.

Study Limitations

Some limitations regarding the influence of other factors, such as stress, need to be considered in our nonhuman animal model of sleep deprivation, and more accurate measurements of sperm quality and quantity as well as mechanistic approaches focused on the mechanisms underlying the findings are desirable. For example, treatment with T supplementation, glucocorticoid inhibition, or NOS modulation during SD would be an elegant way to understand whether these pathways are the key factors for the sleep-fertility relationship in a cause-effect direction. Importantly, although our study has relevance to human conditions, the translational validity of even statistically significant findings are not directly applicable to the clinical framework without clinical studies.

CONCLUSION

Male sexual behavior depends on the interactions of several factors within a complex cascade of chemical events involving hormones, NOS increase, and environmental contexts. The present study demonstrates that lack of sleep is detrimental to sexual behavior and could interfere in the reproductive system of male rats, the latter being clearly reflected in the reduction of spermatic viability and increased expression of apoptosis-related NO genes in the testis. Such findings draw our attention to previously unknown consequences of SD and may be a harbinger of consequences yet to be discovered.

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SUPPLEMENTAL TABLE 1

Effect of paradoxical sleep deprivation (PSD) or sleep restriction (SR) on sperm concentration, pH, volume, viability and mobility parameters of sperm analysis according to type of movement: fast progressive (A), slow progressive (B), in situ (C), or quit (D).

		Mobility parameter						
Variable	**Concentration**	**A**	**B**	**C**	**D**	**pH**	**Volume (mL)**	**Viability (%)**
CTRL	110.5 ± 13.0	36.4 ± 5.7	12.4 ± 2.6	13.4 ± 3.1	36.0 ± 8.4	9.9 ± 0.5	0.4 ± 0.1	69.2 ± 2.4
PSD	93.9 ± 14.5	10.4 ± 2.6*	14.2 ± 3.8	13.8 ± 5.2	45.4 ± 6.8	8.8 ± 0.4	0.2 ± 0.0	36.4 ± 1.0*
SR	99.6 ± 13.8	11.1 ± 4.5*	7.4 ± 2.3	10.3 ± 3.6	37.4 ± 10.2	9.7 ± 0.4	0.4 ± 0.1	41.8 ± 7.0*

* Denotes a significant difference ($P < .01$) compared to a control (CTRL) group.

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