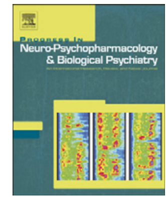
Progress in Neuro-Psychopharmacology & Biological Psychiatry 33 (2009) 562–567

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp



Distinct effects of acute and chronic sleep loss on DNA damage in rats

M.L. Andersen [a,1], D.A. Ribeiro [b,*,1], C.T. Bergamaschi [b,c], T.A. Alvarenga [a], A. Silva [a], A. Zager [a], R.R. Campos [c], S. Tufik [a]

[a] Department of Psychobiology, Universidade Federal de São Paulo (UNIFESP), Brazil
[b] Department of Biosciences, Universidade Federal de São Paulo (UNIFESP), Brazil
[c] Department of Cardiovascular Physiology, Universidade Federal de São Paulo (UNIFESP), Brazil

ARTICLE INFO

Article history:
Received 12 January 2009
Received in revised form 20 February 2009
Accepted 20 February 2009
Available online 28 February 2009

Keywords:
Chronic sleep deprivation
Corticosterone
DNA damage
Rats
Rebound
Single cell gel (comet) assay

ABSTRACT

The aim of this investigation was to evaluate genetic damage induced in male rats by experimental sleep loss for short-term (24 and 96 h) and long-term (21 days) intervals, as well as their respective recovery periods in peripheral blood, brain, liver and heart tissue by the single cell gel (comet) assay. Rats were paradoxically deprived of sleep (PSD) by the platform technique for 24 or 96 h, or chronically sleep-restricted (SR) for 21 days. We also sought to verify the time course of their recovery after 24 h of rebound sleep. The results showed DNA damage in blood cells of rats submitted to PSD for 96 h. Brain tissue showed extensive genotoxic damage in PSD rats (both 24 and 96 h), though the effect was more pronounced in the 96 h group. Rats allowed to recover from the PSD-96 h and SR-21 days treatments showed DNA damage as compared to negative controls. Liver and heart did not display any genotoxicity activity. Corticosterone concentrations were increased after PSD (24 and 96 h) relative to control rats, whereas these levels were unaffected in the SR group. Collectively, these findings reveal that sleep loss was able to induce genetic damage in blood and brain cells, especially following acute exposure. Since DNA damage is an important step in events leading to genomic instability, this study represents a relevant contribution to the understanding of the potential health risks associated with sleep deprivation.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Sleep comprises approximately one-third of a person's lifetime, but its impact on health and medical conditions remains partially unrecognized. Most sleep disorders, such as sleep apnea and insomnia, lead to sleep deprivation. Sleep deprivation disrupts vital biological processes necessary for cognitive function and physical health (Leibowitz et al., 2006), yet the ways in which the body is compromised are not fully understood. Sleep deprivation also represents a common type of stress that can have harmful physiological consequences, possibly leading to death, in experimental animals (Rechtschaffen et al., 1983). In humans, acute sleep deprivation and accumulated sleep debt have been linked to health problems, including metabolic and cardiovascular disease. Sleep deprivation has also been shown to be an independent risk factor for diabetes and associated with an increased risk for hypertension. Short sleep duration is associated with increased mortality (Wolk et al., 2005).

In animal models, studies utilizing sleep deprivation have been conducted to identify the mechanisms involved in the regulation and maintenance of sleep patterns. Indeed, in rats, sleep deprivation induced by the platform technique involves numerous awakenings, which predominantly affect the paradoxical stage of sleep. Currently, most sleep deprivation occurs in the REM/paradoxical phase, which transpires during the last half of the total sleep session. Therefore, this procedure may mimic sleep fragmentation due to repeated awakenings, and thus is a useful tool to investigate the effects of sleep loss. It is well documented that paradoxical sleep deprivation (PSD) in experimental animals results in behavioral (Tufik et al., 1978; Frussa-Filho et al., 2004; Andersen et al., 2002, 2003a,b,c, 2004a, 2005b; Fukushiro et al., 2007; Perry et al., 2008), hormonal (Andersen et al., 2004a, 2005a), immunological (Everson, 2005; Zager et al., 2007; Ruiz et al., 2007) and neurochemical changes (Perry et al., 2008). Notwithstanding, PSD for 24 h was able to increase blood pressure as compared to control rats and led to impairment of the baroreflex control of the cardiovascular system (Sebastiao et al., in preparation). Such responses may be involved in the increased cardiovascular risks of sleep-restricted subjects. Another strategy used in laboratory animals to mimic human-type sleep loss is sleep restriction (SR), in which animals are submitted to gradual loss of sleep over long-term periods. This represents an important model of

Abbreviations: EDTA, ethylenediamine tetraacetic acid; HCl, chlorhydric acid; MMS, methylmethasulfonate; NaCl, sodium chloride; PS, paradoxical sleep; PSD, paradoxical sleep deprivation; REM, rapid eye movement; SR, sleep restriction; SWS, slow wave sleep; Tris, 2-Amino-2-hydroxymethyl-propane-1,3-diol.

* Corresponding author. Departamento de Biociências, Av. Ana Costa, 95 – Vila Mathias 11060-001, Santos, SP, Brazil. Tel.: +55 13 32218058; fax: +55 13 32232592.
E-mail address: daribeiro@unifesp.br (D.A. Ribeiro).

[1] The first two authors contributed equally to this study.

0278-5846/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2009.02.014

sleep consolidation and adaptation. In both protocols, when allowed to sleep, animals compensated for the loss of sleep by spending more time in paradoxical sleep (Machado et al., 2005; Andersen et al., 2008). However, hormonal profile (Andersen et al., 2005a) and estrous cycle disruptions in females (Antunes et al., 2006) remained altered for up to ten days after the termination of the sleep deprivation period. This indicates that sleep loss may promote deleterious and long-lasting effects on certain physiological processes.

A comprehensive understanding of the effects of sleep loss for acute and prolonged periods might shed light on the possibly detrimental impact on the brain as well as other organs. Recently, it has been reported that sleep deprivation affected the expression of genes related to metabolic processes, response to stimulus (including stress and inflammation), circadian/sleep cycles, regulation of cell proliferation and signaling pathways (Cirelli et al., 2004, 2006; Terao et al., 2006). Moreover, it has been demonstrated, that lymphocytes isolated from obstructive sleep apnea patients had higher basal levels of DNA damage and were more sensitive to the effects of the DNA-damaging agents (Kontogianni et al., 2007). However, to our knowledge, no study has investigated the influence of different periods of sleep deprivation on the genome at the level of the single cell in rats. The single cell gel (comet) assay is a rapid, simple and reliable biochemical method for evaluating DNA damage in mammalian cells (Tice et al., 2000). During the procedure, cells with damaged DNA resulting from DNA strand breaks and alkali-labile lesions such as abasic sites or incomplete repair sites display increased DNA migration towards the anode (Singh et al., 1988). Broken DNA migrates farther in the electric field so that the cell resembles a 'comet' with a brightly fluorescent head and a tail region (Olive et al., 1990). The extent of the comet is correlated with increased DNA damage. These images can be analyzed and compared on a cell-to-cell basis. Our group has consistently demonstrated that the single cell gel (comet) assay is a useful tool for detecting DNA breakage in multiple organs and under different paradigms, such as medium-term carcinogenesis assays, parasitic infections or even following exposure to xenobiotics present in the environment *in vivo* (Leite Ade et al., 2007; Grassi et al., 2007; Ribeiro et al., 2004a,b, 2007). Thus, the aim of this investigation was to examine whether the peripheral blood, brain, liver and heart of male rats are particularly sensitive to DNA damage following short-term PSD and long-term SR, as well as their respective recovery periods.

2. Materials and methods

2.1. Animals

Ninety-day-old male Wistar–Hannover rats were bred and raised in the animal facility of the Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia (CEDEME). The animals were housed in a colony maintained at 22 °C with a 12:12 h light–dark cycle (lights on at 0700 h) and allowed free access to food and water inside standard polypropylene cages. Rats used in this study were maintained and treated in accordance with the guidelines established by the protocol for the Care and Use of Laboratory Animals.

2.2. Paradoxical sleep deprivation (PSD)

The PSD procedure consisted of placing rats in a tiled water tank ($143 \times 41 \times 30$ cm) for 24 or 96 h. The tank contained fourteen platforms (6.5 cm in diameter), rising 1 cm above the water surface, thus allowing the rats to move around by leaping from one platform to another. At the onset of each paradoxical sleep (PS) episode, the animal experiences a loss of muscle tonus and falls into water, thereby being abruptly awakened. As our intention was to analyze the alterations caused by total suppression of PS over 24 h (PSD-24 h) or 96 h (PSD-96 h) intervals, we employed the multiple platform procedure, which is well-documented to be effective in producing a total suppression of PS. It therefore seems appropriate to consider these animals as being PS-deprived rather than being completely deprived of sleep. The cage control group was maintained in the same room as the experimental rats for the duration of the study and showed normal sleep patterns, including PS, slow wave sleep (SWS) and wake. Throughout the study, the experimental room was maintained at a controlled temperature and light–dark cycle. Food and water were available *ad libitum*, with chow pellets and water bottles provided on a grid located on top of the tank. The water in the tank was changed daily throughout the PSD period.

2.3. Sleep Restriction (SR)

The SR protocol consisted of submitting the rats to the modified multiple platform method as described above for 18 h intervals (beginning at 1600 h) over 21 days (SR period). After each 18 h sleep deprivation period, the rats were allowed to sleep for 6 h (sleep window beginning at 1000 h). Throughout the SR procedure, rats slept an average of 30–40% of the time, corresponding to 8–9 h per day. This time interval (1000 h to 1600 h) was chosen because it is when PS attains its highest level, as previously described by our group. In both protocols (PSD and SR) PS is significantly compromised.

2.4. Experimental procedure

Rats were randomly distributed into four groups: PSD, SR, recovery and home-cage groups. These groups were subdivided into a total of seven groups ($n = 7$ per group). The PSD groups, PSD-24 h and PSD-96 h were subjected to PSD for 24 h and 96 h, respectively. The SR group, SR-21d, was subjected to SR for 21 days. The recovery groups were sleep-deprived or sleep-restricted and then returned to home-cages and allowed undisturbed, spontaneous sleep for 24 h. These groups were named PSD-24R, PSD-96R and SR-21R. These time-points were chosen based on our previous studies (Andersen et al., 2003c, 2005b; Zager et al., 2007). Finally, the control rats were maintained in separate cages in the same room as the experimental rats during PSD, SR and recovery procedures, and were euthanized on the same day as the other groups. By housing all groups in the same room, we controlled for the environmental conditions. After residing in the water tanks (PSD and SR groups) or home-cages (control and recovery groups), rats were brought to an adjacent room and decapitated between 0900 h and noon with minimum discomfort.

2.5. Sample collection

At the end of the experimental period, the rats were decapitated in a rapid procedure, carried out less than 1 min after removal from the home cage. Blood was collected into sterile tubes containing liquid EDTA. An aliquot of blood was centrifuged at 4 °C for 15 min at 3000 rpm. The serum and plasma was frozen at −80 °C until assays were conducted. In addition, central fragments from heart, liver, right/left kidneys and brain were collected and minced in 0.9% NaCl. The supernatant was removed and the cellular suspensions (~10 µl) were used for the single cell gel (comet) assay.

2.6. Single cell gel (comet) assay

The protocol used for peripheral blood, liver, heart and brain cells followed the guidelines outlined by Sasaki et al. (2002) with some modifications. Briefly, a volume of 5 µl of peripheral blood was added to 120 µl 0.5% low-melting-point agarose at 37 °C, layered onto a pre-coated slide with 1.5% regular agarose, and covered with a coverslip. Similarly, the supernatants (cellular suspension, 10 µl) of the liver, heart, and brain samples were added to 120 µl 0.5% low-melting-point agarose at 37 °C, layered onto a pre-coated slide with 1.5% regular

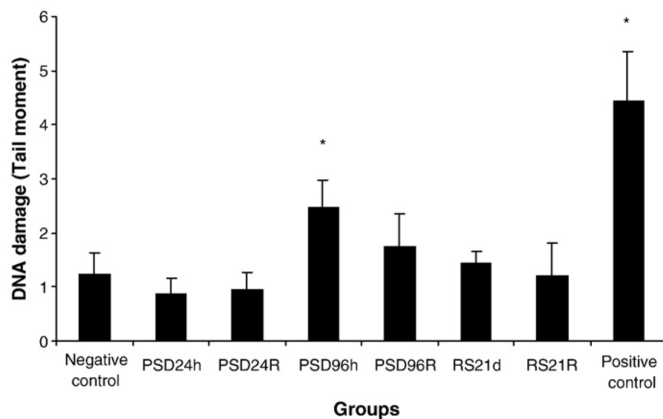


Fig. 1. DNA damage expressed as the mean tail moment in rat blood cells following sleep deprivation. Values are expressed as mean ± SD. $^*p<0.05$ as compared to negative control.

agarose and covered with a coverslip. After brief agarose solidification in a refrigerator, the coverslip was removed and slides were immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl buffer, pH 10, 1% sodium sarcosinate with 1% Triton X-100 and 10% DMSO), for about 1 h. Prior to electrophoresis, the slides were left in alkaline buffer (pH > 13) for 20 min and then electrophoresed for another 20 min, at 0.7 V/cm, 300 mA. After electrophoresis, the slides were neutralized in 0.4 M Tris–HCl (pH 7.5), fixed in absolute ethanol and stored until analysis on a fluorescent microscope at 400 × magnification. Independent positive controls using cells from peripheral blood, liver, brain and heart were treated *in vitro* with 10 µg/mL MMS (methyl-methasulfonate) for 30 min at 37 °C, in order to ensure reproducibility and sensitivity of assay.

2.7. Genotoxicity data analysis

A total of 50 randomly captured comets per animal (25 cells from each slide) (Hartmann et al., 2003) were examined blindly by one expert observer at 400× magnification using a fluorescent microscope (Olympus) connected through a black and white camera to an image analysis system (Comet Assay II, Perceptive Instruments, Suffolk, Haverhill, UK) calibrated previously according to manufacturer's instructions. The computerized image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components, and then evaluates the range of derived parameters. Undamaged cells have an intact nucleus without a tail and damaged cells have the appearance of a comet. To measure DNA damage, two image analysis system parameters were considered: tail intensity (% migrated DNA) and tail moment (the product of the tail length and the fraction of DNA in the comet tail) (Hartmann et al., 2003). Since none of the groups showed significant differences between these parameters, we chose tail moment for the presentation of the results.

2.8. Plasma corticosterone

Plasma corticosterone concentrations were assayed by a double antibody radioimmunoassay method specific for rats and mice using a commercial kit (MP Biomedicals, NY, USA). The sensitivity of the assay was 0.25 ng/ml.

2.9. Statistical methods

The results obtained in the single cell gel (comet) assay were evaluated statistically with the Kruskal–Wallis non-parametric test followed by post-hoc Dunn's test using Sigma Stat for Windows (Jadel Scientific, USA). As PSD, RS and recovery were independent groups, plasma corticosterone concentrations were individually compared to the controls with one-way ANOVA followed by the Duncan test. The level of significance was set at 5%.

3. Results

3.1. Comet assay

In this study, we were able to evaluate genetic damage induced by sleep deprivation *in vivo* in different target organs. Statistically significant differences ($p<0.05$) in DNA damage were found in the blood cells of rats submitted to PSD for 96 h as compared to negative controls, i.e. specimens not exposed to any sleep deprivation or restriction (Fig. 1).

Regarding the brain, extensive genotoxic effects were noticed, in which an increased DNA migration rate was detected in rats suffering PSD for 24 or 96 h. This effect was more pronounced in the PSD-96 h group (Fig. 2). In addition, recovery groups from the PSD-96 h and SR-21 d treatments showed DNA damage when compared to negative controls (Fig. 3). On the other hand, the heart did not show any genotoxicity in any experimental group when compared to the negative control (Fig. 4). The same lack of effect was noted in liver cells, i.e., no statistically significant differences ($p>0.05$) were detected between any experimental group and the negative controls. These findings are summarized in Fig. 3. Rat blood, liver, brain and heart cells were further assayed with MMS to ensure the sensitivity of the assay. A clear sensitivity was observed ($p<0.05$) when compared to negative controls. No animal died unexpectedly during this experiment. Fig. 5 shows an undamaged rat blood cell from a negative control, a liver cell exposed to 96 h of PSD, and an MMS-induced comet cell (positive control).

3.2. Corticosterone concentrations

Corticosterone values are presented in Fig. 6. The ANOVA test showed a strong effect of PSD treatment on corticosterone levels [$F(6,33)=6.89$; $p<0.0001$]. The Duncan test revealed that rats subjected to PSD (24 h, $p<0.01$ and 96 h, $p<0.001$) exhibited higher concentrations of corticosterone than control rats. After 24 h of recovery, corticosterone concentrations returned to baseline (control) concentrations. Chronic SR did not affect corticosterone concentrations ($p>0.05$). There were no significant differences between control and recovery groups.

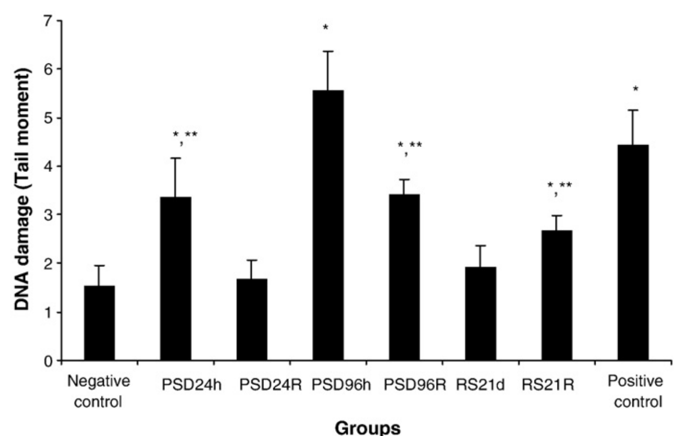


Fig. 2. DNA damage expressed as the mean tail moment in rat brain cells following sleep deprivation. Values are expressed as mean ± SD. $^*p<0.05$ as compared to negative control; $^{**}p<0.05$ as compared to PSD-96 h.

4. Discussion

The present study was undertaken to evaluate genetic damage induced by experimental sleep loss under acute or chronic conditions. The investigation was conducted using the single cell gel (comet) assay. The major finding of the present study was that DNA damage was preferentially induced in the brain and blood cells, whereas no detectable changes were observed in liver or heart cells. To the best of our knowledge, the approach has not been demonstrated before.

The results of this study displayed that the alkaline single cell gel (comet) assay in our experimental conditions was able to detect the presence of DNA damage in peripheral blood cells of rats submitted to sleep deprivation for 96 h. It seems that 24 h (acute exposure) or 21 days (chronic exposure) of sleep deprivation were not sufficient for a positive genotoxic response in peripheral blood cells. By comparison, a study conducted by Kontogianni et al. (2007) have evidenced higher basal levels of DNA damage and were more sensitive to the effects of the DNA-damaging agents such as hydrogen-peroxide, ethanol and gamma-irradiation in lymphocytes isolated from obstructive sleep apnea patients. Some authors have postulated that the effects of sleep loss on cellular and genomic activities could contribute to inflammatory cytokine activity. For instance, it has been reported that monocyte production of interleukin-6 and tumor necrosis factor alpha were significantly greater in the morning after a night of sleep loss, compared with morning levels following uninterrupted sleep (Irwin et al., 2006). These data were confirmed in a recent study conducted by the same research group (Irwin et al., 2008). Inflammation processes are associated with increased risk of some degenerative diseases such as cardiovascular disorders, arthritis, hypertension and diabetes mellitus. This link is attributed to the fact that although such mechanisms help to eradicate several pathological conditions, they inevitably expose target organs to certain endogenous genotoxic agents (Fitzpatrick, 2001). Taken as a whole, our findings support the notion that PSD over 96 h was able to induce DNA damage in blood cells.

Given that the brain is considered to be the main organ affected by sleep deprivation, we carefully evaluated the brains of rats subjected to the sleep deprivation protocols. Our results demonstrated extensive genotoxic damage in rats exposed to PSD for 24 h and 96 h, in a time-dependent fashion, as the effect was more pronounced in the 96 h group. This result is in agreement with our previous observation that more dramatic changes occur after four days of PSD (Andersen et al., 2003c, 2005b; Zager et al., 2007), and suggests that long periods of PSD may lead to long-term effects that may become increasingly harmful.

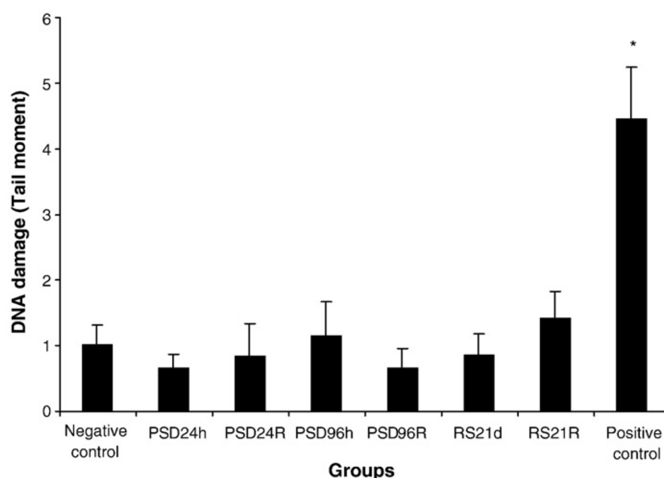


Fig. 3. DNA damage expressed as the mean tail moment in rat liver cells following sleep deprivation. Values are expressed as mean ± SD. *$p<0.05$ as compared to negative control.

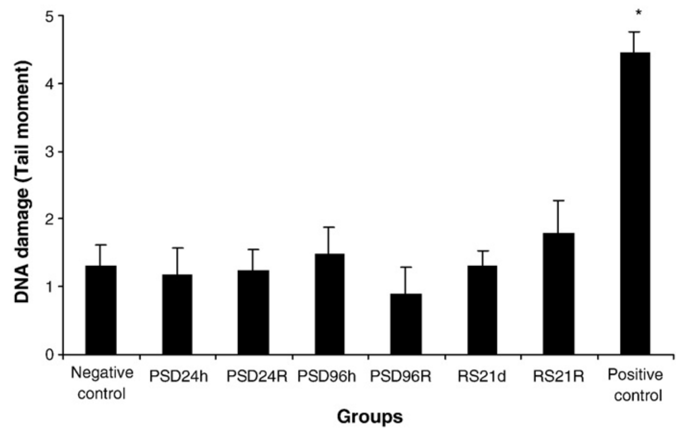


Fig. 4. DNA damage expressed as the mean tail moment in rat heart cells following sleep deprivation. Values are expressed as mean ± SD. *$p<0.05$ as compared to negative control.

Although different protocols of long-term SR could be employed (Koban et al., 2006), most likely they would produce the same results, as both induce suppression of paradoxical sleep. Indeed, our protocol of SR led to a complete suppression of paradoxical sleep which persisted throughout the whole restriction period (Machado et al., 2005). We acknowledge that difference in the degree of paradoxical sleep reduction between the acute and chronic experiments could led to distinct effects over the organs since PSD resulted in more marked alteration in relation to SR. Thus, the duration of the sleep loss might have different implication on the overall physiology on the organism's biological functions.

It is important to keep in mind that the experimental model has stress as an inherent component. Herein, corticosterone concentrations were increased after PSD, whereas the SR group was unaffected, as was expected and previously reported (Zager et al., 2007). Although the basal concentrations of corticosterone could be deemed as somewhat elevated, this only emphasizes the notion that all rats were manipulated in the very same way, regardless of what group they belonged to. The values found here reflect the hormone concentrations of the rat strain investigated as well as the effects of the experimental conditions that the rats experienced. The increase found in corticosterone during PSD showed that the rats were adequately responsive to this condition. The chronically sleep-restricted rats seem to have coped with the stress during the 21-day period.

Recent studies suggest that the nitric oxide mechanism is closely involved in the sleep pattern (Schutz et al., 2004) as well as in sleep deprivation-induced behavioral alterations in rats (Andersen et al., 2007) and oxidative damage in mice (Kumar and Garg, 2008; Kumar and Singh, 2008). Nitric oxide plays an important role in host defense and homeostasis when generated at a low level for a brief period of time (Beevi et al., 2004), whereas the prolonged induction of nitric oxide levels may contribute to a variety of pathological phenomena associated with inflammatory processes and genomic instability (Nathan and Xie, 1994). This is because nitric oxide is a potent free radical able to induce oxidative stress and, in turn, DNA damage via mutagenesis (Nakamura et al., 2006). The consensus is that DNA damage can be caused by multiple factors such as oxidative stress (Kan et al., 2002) as well as other exogenous and/or endogenous sources (Demirbag et al., 2005). On the other hand, a study conducted by Cirelli et al. (1999) argued against the hypothesis that sustained waking can significantly damage brain cells through excitotoxic or oxidative mechanisms and that massive cell death may explain the fatal consequences of sleep deprivation.

No DNA damage was noted in the recovery group (PSD-24R) after 24 h of PSD. Therefore, we might conclude that damage induced by acute sleep loss can easily be repaired in 24 h of normal sleep.

Fig. 5. Representative comet images of a blood cell from a negative control (a), a liver cell exposed to 96 h of PSD (b), and an MMS-treated cell (positive control) (c). DNA was stained with ethidium bromide; 40× magnification.

However, animals submitted to the recovery group from the 96 h PSD treatment still presented DNA damage in the brain, although there was a trend toward normalization. It is clear that repair systems can be activated to restore DNA damage, but the effectiveness of the system probably depends on the nature and intensity of the insult. Interestingly, our results also revealed that recovery groups from the SR-21 d treatment also showed the presence of DNA damage as compared to negative controls. The single cell gel (comet) assay does not necessarily predict the mutagenic potential of pathological conditions; moreover, the genotoxicity of chronic sleep deprivation associated with subsequent awake periods can be modulated in combination with other DNA-damaging agents that are present in the brain following chronic genotoxic injury at low levels. This could partially explain these results. Taken together, there seems to be evidence that sleep loss can induce genetic damage in brain cells, possibly as a result of oxidative DNA damage.

The *in vivo* single cell gel (comet) assay guidelines (Tice et al., 2000) recommend that liver cells should in particular be analyzed, since the liver is a main organ for metabolism. To date, there is no data in the literature regarding the genotoxic potential of sleep deprivation on liver cells using *in vivo* experimental models. Chang et al. (2008) argued that sleep deprivation predisposes the liver to oxidative stress and phospholipid damage, therefore leading to injury of the genetic apparatus. Nevertheless, our results with the single cell gel (comet) assay failed to detect DNA breakage in liver cells. However, it is not yet clear how and/or when sleep deprivation might exert these biological actions in this metabolic organ, since the development of genetic damage in target cells depends not only on the initial levels of induced DNA damage and its repair, but also on other contributing factors, including the production of reactive metabolites, their distribution, and their effects on cell proliferation. Furthermore, *in vitro* and *in vivo* genotoxicity tests detect compounds that induce genetic damage directly or indirectly by various mechanisms. Nevertheless, no single test is capable of detecting all genotoxic agents. Thus, for a more detailed judgment on the genotoxic potential of sleep deprivation on liver cells, a battery of tests may be necessary.

To further elucidate the possible outcomes of sleep deprivation on the cardiovascular system, we evaluated genotoxicity in the heart as well as the blood. Loss of sleep induces elevations in circulating levels of cholesterol (Andersen et al., 2004b), stress-related hormones (Andersen et al., 2004a) and catecholamines (Irwin et al., 1999, 2003; Andersen et al., 2005b), with attendant increases of blood pressure and heart rate (Kato et al., 2000). Furthermore, habitual sleep loss and insomnia are markers of subclinical heart disease and are independent predictors of cardiovascular disease risk, particularly in males (Mallon et al., 2002). Our results show that neither acute nor chronic sleep deprivation exerted any detectable genotoxic activity in the heart. As these findings are novel and the underlying mechanisms are still unknown, it is difficult to satisfactorily explain these observations. Independent of a biological mechanism involved in this phenomenon, it seems that sleep deprivation does not exert noxious activity on heart cells, specifically related to genotoxic damage. However, we could speculate that despite the lack of genotoxic damage in heart cells, changes in the central nervous system may trigger cardiovascular dysfunction. Further studies are warranted to elucidate the issue.

In conclusion, our results reveal that sleep deprivation exerted genetic damage in the form of DNA breakage in blood and brain cells, especially following acute exposure. Since DNA damage is an important step in events leading to genomic instability that can later promote the development of degenerative diseases, this study represents a relevant contribution to the evaluation of the potential health risks associated with sleep deprivation.

Acknowledgements

The authors gratefully acknowledge the invaluable assistance of Waldemarks Leite and Darine Villela. This work was supported by grants from Associação Fundo de Incentivo à Psicofarmacologia (AFIP) and FAPESP (#06/58274-5 to T.A.A., 2007/01228-4 to A.Z., 07/56620-6 to A.S., and CEPID no. 98/14303-3 to S.T). MLA, DAR, CRTB, RC and ST are recipients of the CNPq fellowship.

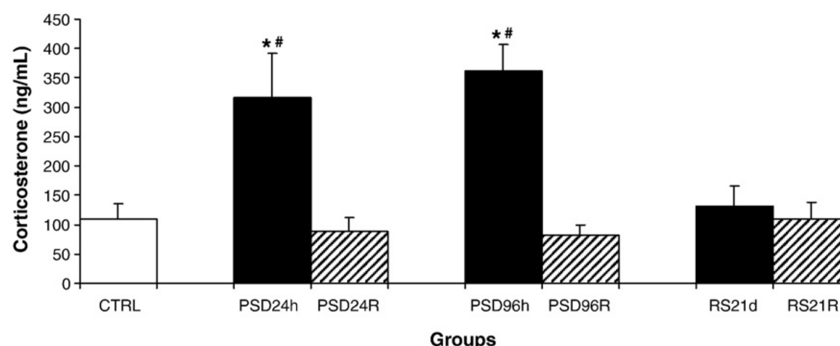


Fig. 6. Corticosterone concentrations in the home-cage control group (CTRL), the paradoxical sleep-deprived (PSD) for 24 and 96 h groups, the sleep-restricted for 21 days group (SR), and their associated recovery (R) groups. *Different from control group; #Different from respective recovery group. Values are expressed as mean ± SEM.

References

Andersen ML, Bignotto M, Machado RB, Tufik S. Does paradoxical sleep deprivation and cocaine induce penile erection and ejaculation in old rats? Addict Biol 2002;7:285–90.

Andersen ML, Bignotto M, Tufik S. Cocaine-induced genital reflexes during paradoxical sleep deprivation and recovery. Physiol Behav 2003a;78:255–9.

Andersen ML, Bignotto M, Tufik S. The effect of apomorphine on genital reflexes in male rats deprived of paradoxical sleep. Physiol Behav 2003b;80:211–5.

Andersen ML, Bignotto M, Tufik S. Facilitation of ejaculation after methamphetamine administration in paradoxical sleep deprived rats. Brain Res 2003c;978:31–7.

Andersen ML, Bignotto M, Tufik S. Hormone treatment facilitates penile erection in castrated rats after sleep deprivation and cocaine. J Neuroendocrinol 2004a;16:154–9.

Andersen ML, Martins PJ, D'Almeida V, Santos RF, Bignotto M, Tufik S. Effects of paradoxical sleep deprivation on blood parameters associated with cardiovascular risk in aged rats. Exp Gerontol 2004b;39:817–24.

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 2005a;14:83–90.

Andersen ML, Perry JC, Tufik S. Acute cocaine effects in paradoxical sleep deprived male rats. Prog Neuropsychopharmacol Biol Psychiatry 2005b;29:245–51.

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;31:652–7.

Andersen ML, Antunes IB, Silva A, Alvarenga TA, Baracat EC, Tufik S. Effects of sleep loss on sleep architecture in Wistar rats: gender-specific rebound sleep. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:975–83.

Antunes IB, Andersen ML, Baracat EC, Tufik S. The effects of paradoxical sleep deprivation on estrous cycles of the female rats. Horm Behav 2006;49:433–40.

Beevi SS, Rasheed AM, Geetha A. Evaluation of oxidative stress and nitric oxide levels in patients with oral cavity cancer. Jpn J Clin Oncol 2004;34:379–85.

Chang HM, Mai FD, Chen BJ, Wu UI, Huang YL, Lan CT, et al. Sleep deprivation predisposes liver to oxidative stress and phospholipid damage: a quantitative molecular imaging study. J Anat 2008;212:295–305.

Cirelli C, Shaw PJ, Rechtschaffen A, Tononi G. No evidence of brain cell degeneration after long-term sleep deprivation in rats. Brain Res 1999;840:184–93.

Cirelli C, Gutierrez CM, Tononi G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron 2004;41:35–43.

Cirelli C, Faraguna U, Tononi G. Changes in brain gene expression after long-term sleep deprivation. J Neurochem 2006;98:1632–45.

Demirbag R, Yilmaz R, Erel O, Gultekin U, Asci D, Elbasan Z. The relationship between potency of oxidative stress and severity of dilated cardiomyopathy. Can J Cardiol 2005;21:851–5.

Everson CA. Clinical assessment of blood leukocytes, serum cytokines, and serum immunoglobulins as responses to sleep deprivation in laboratory rats. Am J Physiol Regul Integr Comp Physiol 2005;289:R1054-1063.

Fitzpatrick FA. Inflammation, carcinogenesis and cancer. Int Immunopharmacol 2001;1:1651–67.

Frussa-Filho R, Goncalves MT, Andersen ML, de Araujo NP, Chinen CC, Tufik S. Paradoxical sleep deprivation potentiates amphetamine-induced behavioural sensitization by increasing its conditioned component. Brain Res 2004;1003:188–93.

Fukushiro DF, Calzavara MB, Trombin TF, Lopez GB, Abilio VC, Andersen ML, et al. Effects of environmental enrichment and paradoxical sleep deprivation on open-field behavior of amphetamine-treated mice. Physiol Behav 2007;92:773–9.

Grassi TF, Camargo EA, Salvadori DM, Marques ME, Ribeiro DA. DNA damage in multiple organs after exposure to chlorhexidine in Wistar rats. Int J Hyg Environ Health 2007;210:163–7.

Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, et al. Recommendations for conducting the in vivo alkaline Comet assay. 4th International Comet Assay Workshop. Mutagenesis 2003;18:45–51.

Irwin M, Thompson J, Miller C, Gillin JC, Ziegler M. Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in humans: clinical implications. J Clin Endocrinol Metab 1999;84:1979–85.

Irwin M, Clark C, Kennedy B, Christian Gillin J, Ziegler M. Nocturnal catecholamines and immune function in insomniacs, depressed patients, and control subjects. Brain Behav Immun 2003;17:365–72.

Irwin MR, Wang M, Campomayor CO, Collado-Hidalgo A, Cole S. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. Arch Intern Med 2006;166:1756–62.

Irwin MR, Wang M, Ribeiro D, Cho HJ, Olmstead R, Breen EC, et al. Sleep loss activates cellular inflammatory signaling. Biol Psychiatry 2008;64:538–40.

Kan E, Undeger U, Bali M, Basaran N. Assessment of DNA strand breakage by the alkaline COMET assay in dialysis patients and the role of Vitamin E supplementation. Mutat Res 2002;520:151–9.

Kato M, Phillips BG, Sigurdsson G, Narkiewicz K, Pesek CA, Somers VK. Effects of sleep deprivation on neural circulatory control. Hypertension 2000;35:1173–5.

Koban M, Le WW, Hoffman GE. Changes in hypothalamic corticotropin-releasing hormone, neuropeptide Y, and proopiomelanocortin gene expression during chronic rapid eye movement sleep deprivation of rats. Endocrinology 2006;47:421–31.

Kontogianni K, Messini-Nikolaki N, Christou K, Gourgoulianis K, Tsilimigaki S, Piperakis SM. DNA damage and repair capacity in lymphocytes from obstructive sleep apnea patients. Environ Mol Mutagen 2007;48:722–7.

Kumar A, Garg R. A role of nitric oxide mechanism involved in the protective effects of venlafaxine in sleep deprivation. Behav Brain Res 2008;194:169–73.

Kumar A, Singh A. Possible nitric oxide modulation in protective effect of (Curcuma longa, Zingiberaceae) against sleep deprivation-induced behavioral alterations and oxidative damage in mice. Phytomedicine 2008;15:577–86.

Leibowitz SM, Lopes MC, Andersen ML, Kushida CA. Sleep deprivation and sleepiness caused by sleep loss. Sleep Med Clin 2006;1:31–45.

Leite Ade L, Santiago Jr JF, Levy FM, Maria AG, Fernandes Mda S, Salvadori DM, et al. Absence of DNA damage in multiple organs (blood, liver, kidney, thyroid gland and urinary bladder) after acute fluoride exposure in rats. Hum Exp Toxicol 2007;26:435–40.

Machado RB, Suchecki D, Tufik S. Sleep homeostasis in rats assessed by a long-term intermittent paradoxical sleep deprivation protocol. Behav Brain Res 2005;160:356–64.

Mallon L, Broman JE, Hetta J. Sleep complaints predict coronary artery disease mortality in males: a 12-year follow-up study of a middle-aged Swedish population. J Intern Med 2002;251:207–16.

Nakamura Y, Yasuoka H, Tsujimoto M, Yoshidome K, Nakahara M, Nakao K, et al. Nitric oxide in breast cancer: induction of vascular endothelial growth factor-C and correlation with metastasis and poor prognosis. Clin Cancer Res 2006;12:1201–7.

Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. Cell 1994;78:915–8.

Olive PL, Banath JP, Durand RE. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet" assay. Radiat Res 1990;122:86–94.

Perry JC, D'Almeida V, Antunes IB, Tufik S. Distinct behavioral and neurochemical alterations induced by intermittent hypoxia or paradoxical sleep deprivation in rats. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:87–94.

Rechtschaffen A, Gilliland MA, Bergmann BM, Winter JB. Physiological correlates of prolonged sleep deprivation in rats. Science 1983;221:182–4.

Ribeiro DA, Favero Salvadori DM, da Silva RN, Ribeiro Darros B, Alencar Marques ME. Genomic instability in non-neoplastic oral mucosa cells can predict risk during 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. Oral Oncol 2004a;40:910–5.

Ribeiro DA, Pereira PC, Machado JM, Silva SB, Pessoa AW, Salvadori DM. Does toxoplasmosis cause DNA damage? An evaluation in isogenic mice under normal diet or dietary restriction. Mutat Res 2004b;559:169–76.

Ribeiro DA, Calvi SA, Picka MM, Persi E, de Carvalho TB, Caetano PK, et al. DNA damage and nitric oxide synthesis in experimentally infected Balb/c mice with Trypanosoma cruzi. Exp Parasitol 2007;116:296–301.

Ruiz FS, Andersen ML, Zager A, Martins RC, Tufik S. Sleep deprivation reduces the lymphocyte count in a non-obese mouse model of type 1 diabetes mellitus. Braz J Med Biol Res 2007;40:633–7.

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, et al. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res 2002;519:103–19.

Schutz TC, Andersen ML, Tufik S. Influence of temporomandibular joint pain on sleep patterns: role of nitric oxide. J Dent Res 2004;83:693–7.

Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.

Terao A, Wisor JP, Peyron C, Apte-Deshpande A, Wurts SW, Edgar DM, et al. Gene expression in the rat brain during sleep deprivation and recovery sleep: an Affymetrix GeneChip study. Neuroscience 2006;137:593–605.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 2000;35:206–21.

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.

Wolk R, Gami AS, Garcia-Touchard A, Somers VK. Sleep and cardiovascular disease. Curr Probl Cardiol 2005;30:625–62.

Zager A, Andersen ML, Ruiz FS, Antunes IB, Tufik S. Effects of acute and chronic sleep loss on immune modulation of rats. Am J Physiol Regul Integr Comp Physiol 2007;293: R504-509.