THE EFFECTS OF SLEEP DEPRIVATION AND SLEEP RECOVERY ON PAIN THRESHOLDS OF RATS WITH CHRONIC PAIN

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ABSTRACT

The aim of this study was to compare the effects of different paradoxical sleep deprivation (PSD) methods and sleep recovery on the pain threshold of rats submitted to inflammatory and neuropathic pain models. Wistar rats were randomly assigned to adjuvant-induced arthritis (AIA), chronic constrictive injury of sciatic nerve (CCI) and non-handled control group. PSD was performed using small (SP) or large platforms (LP) in the water tank technique. Grid and home-cage groups were also evaluated. Pain threshold was determined in dry environment using the hot plate test, before, during and after (recovery) PSD. The data showed that AIA and CCI differ from control groups from the second day on after pain-inducing procedures and lasted until the third day of sleep recovery. PSD reduced the pain threshold in all groups studied, regardless of the method used. Sleep recovery did not restore the baseline pain threshold in arthritis-induced animals, but did so in the CCI group exposed to SP and LP methods.

Key-words: sleep deprivation, pain, adjuvant-induced arthritis, sciatic nerve constriction, hot plate, rats.

INTRODUCTION

Pain is a factor that has been reported to be a leading cause of insomnia in medical conditions (Moldofsky & Scarisbrick, 1976; Drewes et al., 1994, 1997). Clinical trials using rheumatic and fibromyalgia patients (Drewes et al., 1994; Roizenblatt et al., 2001) and animal studies with experimental models of polyarthritic rats (Landis et al., 1988, 1989; Andersen & Tufik, 2000) confirm the association between painful manifestations and sleep disruption.

Sleep constitutes a dynamic form of homeostasis restoration and it is pertinent to assume that its abolishment may lead to different behavioral alterations, such as increasing pain sensitivity. In fact, some studies report the influence of sleep disturbances on pain sensitivity. Despite this, such influence is not completely understood. Ukponmwan et al. (1984, 1986) reported reduction of antinociceptive property in enkephalinases, morphine and swimming in paradoxical sleep-deprived rats. Onen et al. (2000) described that the threshold of vocalization response to pressure nociceptive stimuli in rats is not reduced by PSD, but it is augmented during the recovery period.

The reciprocal influence between pain and sleep deficits does not seem to be a problem in normal individuals as it vanishes with the cessation of pain. Chronic pain sufferers, however, may develop a positive feedback relationship and aggravate their problems. Studies by Moldofsky et al. (1975), Drewes et al. (1997) and Lentz et al. (1999) suggest that non-efficient sleep produces an increase in pain as well as fatigue in rheumatoid arthritis and fibromyalgia patients. If such increase in pain worsened sleep and enhanced pain even further, or if there were adaptive solutions, many other important issues still remain unanswered. There are, nowadays, some animal models of chronic pain

that seem suitable to answer these questions. Among them, the inflammatory chronic pain of experimental arthritis induced by adjuvant and the neuropathic chronic pain of sciatic nerve constriction may be valuable tools. To use them for such purpose, it is heuristically necessary to demonstrate that they reproduce the clinical observation of increased pain after non-efficient sleep.

The aim of this study was to investigate the effects of PSD methods as well as sleep recovery on the pain threshold of rats submitted to inflammatory and neuropathic pain models.

METHODS

Animals

Adult, male Wistar rats, aged approximately 90 days at the beginning of the study were used. The whole study was conducted under a controlled 12:12h light/dark cycle (lights on at 07:00h) and room temperature ($23 \pm 2°C$). The animals were kept in a quiet room inside plastic cages covered with soft sawdust, with rat chow and water available *ad libitum*. Seven days were allowed for adaptation to housing environment before baseline nociceptive testing.

Ethical Standard

Animal care was in compliance with the recommendations of the Committee for Research and Ethical Issues of IASP (1983) and approved by the Ethics Committee of the Universidade Federal de São Paulo (N. 065/99). The animals were randomly assigned to three groups: Adjuvant-induced arthritis (AIA), Chronic Constrictive Injury (CCI) and Non-manipulated controls (NM).

Adjuvant-induced arthritis

After administration of the anesthetic (140mg/kg de ketamine, i.p.), arthritis was induced in 40 animals by s.c. injection of 0.1ml of Freund adjuvant (complete fraction of denatured *Mycobacterium butyricum* suspended in mineral oil, Sigma Chemical Co., St. Louis, USA) in the right hind limb.

Chronic Constrictive Injury

After onset of ketamine anesthesia (140mg/kg of body weight, i.p.), CCI was produced in 40 rats. The sciatic nerve was exposed to the level of the lateral face of the right posterior limb and 4 ligatures (4.0 chromic catgut) were tied around the common sciatic nerve, so that circulation through the epineural vasculature was not totally interrupted. The procedure was comparable to the original description (Bennet & Xie, 1988).

Study design

The experiment was performed throughout a 9-day period: baseline in dry environment (days 1 and 2), paradoxical sleep deprivation (days 3, 4, 5 and 6) and recovery in dry environment (days 7, 8 and 9). Following the first test, the animals were randomly distributed into three groups (AIA, CCI or CTRL) and chronic pain inducing procedures were performed. Two days after (test 2), pain threshold was measured and the animals were placed in the tank or remained in their home-cages. Daily, during the 4 days of PSD (Tests 3 to 6) and during the 3 recovery days (Tests 7 to 9) the hot plate test was performed. The investigator was blind to the type of manipulation used to induce sleep deprivation.

Paradoxical Sleep Deprivation Procedures

Two methods of PSD procedures were employed using small (6.5cm in diameter) and large (14cm in diameter) platforms. The PSD technique consists of placing ten rats for 96h in a tiled water tank (123 x 44 x 44cm), containing 14 platforms, dipped in water until 1cm of their upper surface. In this method, the animals are capable of moving inside the tank, jumping from one platform to the other. When the animal enters the paradoxical sleep phase, it falls into the water, due to muscle atonia, and wakes up. Since the large platforms (LP) also produce sleep deprivation, a new proposed control group (Suchecki & Tufik, 2000), in which animals are placed onto a grid, was used. The grid (GR) group was placed on a stainless steel wire grid, with segments spaced 2.5 cm from each other. The grid was fixed horizontally at 1cm above the water in the deprivation tank. The cage control group (CTRL) was housed in plastic cages and allowed to sleep normally.

Assessment of nociception

Pain sensitivity to noxious thermal stimuli was assessed between 09:00h and 11:00h. The hot-plate apparatus to test pain threshold consists of a 20-cm diameter metal hot-plate surface set at $50^{o}C$ with a Plexiglas cage that fits onto the hot metal surface, and a foot-switch operated timer. Pain threshold was measured by the latency to nociceptive response (licking of any paw) with a maximum cutoff time of 90 seconds.

Statistical analysis

The data were analyzed using two-way ANOVA for repeated measures with behavioral test and group as main factors, followed by Test of Dunnett as *post hoc* test. The level of significance was set at $p < 0.05$.

The effect of experimental pain models on pain threshold

Two-way ANOVA followed by the test of Dunnett revealed that AIA and CCI differed from control groups from the second day on after pain inducing-procedures (test 2) and lasted until the third day of sleep recovery (test 9).

The effect of PSD methods on experimentally-induced pain models

The control group (non-manipulated animals) showed decreased latency in the hot plate test during the 4 days of PSD and on the first day of rebound using both SP and LP. The GR group also presented a reduction of pain threshold during the PSD period (Table 1).

Table 1: Values of pain threshold of CTRL animals submitted to the different procedures of sleep deprivation throughout the experimental period. Values are expressed in seconds as mean ± s.e.m.

	Cage	Grid	Large Platform	Small Platform
Baseline	53.2 ± 3.7	53.5 ± 2.7	53.1 ± 4.4	53.2 ± 4.6
Pre-PSD	53.1 ± 4.8	53.6 ± 4.6	52.9 ± 3.2	53.0 ± 3.6
PSD-24h	53.4 ± 1.4	48.6 ± 2.5*	47.3 ± 2.5*	37.3 ± 1.9*
PSD-48h	53.4 ± 1.7	46.5 ± 2.7*	43.3 ± 2.9*	35.6 ± 2.5*
PSD-72h	53.2 ± 1.6	43.3 ± 2.9*	40.0 ± 1.1*	32.6 ± 3.1*
PSD-96h	53.1 ± 1.9	40.6 ± 3.2*	37.1 ± 2.4*	29.7 ± 2.8*
R-24h	52.9 ± 2.0	51.4 ± 2.1	48.3 ± 3.5*	44.1 ± 4.4*
R-48h	52.8 ± 1.9	53.0 ± 3.5	51.3 ± 3.0	50.6 ± 6.1
R-72h	53.0 ± 1.8	52.9 ± 3.0	53.0 ± 2.7	53.0 ± 6.6

*Values significantly different from those of the cage group, $p<0.05$ (two-way ANOVA followed by post hoc Test of Dunnett).

Regarding AIA group (Table 2), PSD induced a conspicuous alteration in pain thresholds when animals were sleep-deprived by the SP and LP methods. The latency to paw withdrawal was lowered in SP from the first day of PSD on and remained lower even during the rebound period compared to CTRL group. LP group presented a reduction of paw withdrawal on the second day of PSD and also remained low until the third day of the recovery period. Curiously, regarding the GR group, the pain threshold was significantly higher in the second and third days of sleep rebound compared to CTRL animals.

Table 2: Values of pain threshold of arthritic rats (AIA) animals submitted to the different procedures of sleep deprivation throughout the experimental period. Values are expressed in seconds as mean ± s.e.m.

	Cage	Grid	Large Platform	Small Platform
Baseline	53.7 ± 2.3	53.8 ± 6.7	53 ± 3.1	54.1 ± 1.3
Pre-PSD	42.5 ± 2.3	42.5 ± 3.0	42 ± 1.7	42.9 ± 1.3
PSD-24h	36.2 ± 1.8	40.3 ± 3.2	36.3 ± 6.9	25.1 ± 3.6*
PSD-48h	40.2 ± 1.3	37.8 ± 5.0	29.4 ± 5.7*	24.9 ± 3.3*
PSD-72h	39.0 ± 2.8	34.8 ± 4.0	23.5 ± 4.7*	17.6 ± 9.4*
PSD-96h	38.3 ± 3.2	34.9 ± 4.4	21.2 ± 1.7*	17.0 ± 7.8*
R-24h	38.1 ± 2.5	42.5 ± 2.1	31.2 ± 8.6*	31.1 ± 2.0*
R-48h	39.9 ± 2.0	45.1 ± 2.9*	35.0 ± 7.2*	32.6 ± 2.2*
R-72h	42.7 ± 3.6	46.2 ± 2.6*	37.8 ± 3.9*	32.8 ± 1.9*

* Values significantly different from those of cage control group, $p < 0.05$.

In regard to CCI animals (Table 3), we observed that the exposure to both SP and LP methods resulted in a decrease of the pain threshold during the 96 h of PSD. When placed on the grid, animals exhibited a reduced latency to paw withdrawal on days 3 and 4 of PSD and on the two first days of rebound.

Table 3: Values of pain threshold rats with chronic constrictive injury of the sciatic nerve (CCI) submitted to the different procedures of sleep deprivation throughout the experimental period. Values are expressed in seconds as mean ± s.e.m.

	Cage	Grid	Large Platform	Small Platform
Baseline	53.7 ± 2.7	53.3 ± 3.0	53.5 ± 3.2	54.0 ± 5.5
Pre-PSD	39.4 ± 2.9	39.6 ± 2.3	39.8 ± 1.5	39.0 ± 2.5
PSD-24h	30.7 ± 2.1	34.8 ± 7.9	22.9 ± 8.1*	20.8 ± 6.7*
PSD-48h	33.9 ± 1.7	31.3 ± 5.1	19.8 ± 1.3*	15.7 ± 8.6*
PSD-72h	32.7 ± 1.6	25.0 ± 2.9*	19.0 ± 1.0*	10.8 ± 2.7*
PSD-96h	31.9 ± 1.1	24.2 ± 4.7*	18.8 ± 1.1*	10.3 ± 4.3*
R-24h	30.7 ± 1.3	39.0 ± 5.9*	27.5 ± 2.5	26.0 ± 6.3
R-48h	26.0 ± 1.5	37.0 ± 4.8*	28.7 ± 2.4	30.1 ± 5.5
R-72h	29.5 ± 1.0	36.4 ± 5.7	32.6 ± 3.0	35.0 ± 11.4

* Values significantly different from those of the cage group, $p < 0.05$.

DISCUSSION

Regarding the relevance of sleep and pain, several studies have described sleep disturbances in patients suffering from different pain disorders, and although it seems logical that pain can disturb sleep, sleep disturbances per se may also exacerbate pain (Moldofsky & Scarisbrick, 1976; Kryger & Shapiro, 1992). In the present study, we observed that pain thresholds to thermal noxious stimulation were reduced during PSD in all groups studied, independently of which deprivation method was used. Beside the confirmation that sleep disturbance increases sensibility to pain in experimental animals, this result indicated that chronic pain models may be used as a valuable paradigm to study the reciprocal influences between non-efficient sleep and pain. The results disclosed also some new aspects for investigation. Sleep recovery did not restore baseline pain threshold in arthritic rats, but it did so in CCI group placed on both SP and LP. Additionally, the second day of rebound was sufficient to restore pain threshold to baseline values in control animals. The Grid method induced an increase of pain threshold latency in AIA animals (tests 8 and 9) and a decrease in CCI (tests 5 and 6) and control (tests 3 to 6) groups, leading to the understanding that this method also interferes with pain sensitivity.

The small platform method of PSD induced a greater increase in pain sensitivity in all groups studied comparatively to the large platform. It is well known that large platform does not deprive sleep as much as the small one. In fact, earlier studies tried to use large platform as controls for the PSD carried-out in small platforms, but it was abandoned due to the partial deprivation it promotes. The correlation found between the magnitude of PSD promoted by SP and LP and the level of increase in pain sensitivity indicates the linearity of the effect studied. On the other hand, both models of chronic-pain seem to offer a valuable way to study the pain-sleep relationships. A choice between them may be determined by the differences observed in the rebound period or other details as the observed in the grid method.

The mechanism by which PSD and sleep recovery modifies the pain thresholds has not been completely established until now. Neurotransmitter systems, such as serotonergic and opioidergic pathways have been involved in the participation of pain and sleep manipulation (Onen et al., 2000, 2001). Concerning sleep manipulations, an inverse relationship between brain serotonergic activity and pain has been reported in several animal studies. These studies have shown increased pain responsiveness employing neural lesions and pharmacological depletion of brain serotonin (Tenen, 1967). Therefore, PSD appears to increase the rate of serotonin metabolism in the rat brain (Youngblood et al., 1997).

Ukponmwan et al. (1984, 1986) reported that 96h of PSD abolish the antinociceptive effects of analgesic compounds such as phosphoramidon (an enkephalinase inhibitor) and morphine in the rat brain. These findings are consistent with the hypothesis that animals deprived of paradoxical sleep might have smaller responsiveness of opioid receptors to endogenous enkephalines (Onen et al., 2000). Kay (1975) demonstrated that during chronic administration of morphine, paradoxical sleep time persistently decreases, suggesting that the chronic use of this analgesic produces PSD. Thus, the tolerance that takes place with chronic use of some analgesics may be mediated, in part, by PSD-induced reductions in pain threshold (Hicks et al., 1979).

Notwithstanding the exact mechanism responsible for the relationship between sleep restriction and pain sensitivity remains unknown, the results support the occurrence of a vicious, self-perpetuating and non-restorative cycle of pain, PSD and anxiety, as advanced previously by Phillips & Cousins (1986). If pain remains unrelieved for several days, then patients would suffer of anger and depression, which also contribute to the vicious circle as patients become demoralized and lose confidence in the ability of their medical attendants to relieve their pain. Moreover, the sleep disturbance participates in the problem (Phillips & Cousins, 1986). The psychological component has the potential to interact with both pain and sleep further complicating the situation. The present demonstration that rats under chronic pain and submitted to PSD may be used to approach this vicious circle seems to be a promising idea.

Finally, one may considerer that the reciprocal relationship of pain sensitivity and sleep is not fortuitous. Pain is an important evolutionary acquisition that granted survival by its role to warn the occurrence of some dangerous or noxious process in the organism. To be awake in such situations seems adaptive. Inversely, as sleep deprivation induces somnolence and lowers attention, an increase in pain sensitivity seems to compensate them and grant wakefulness. Such considerations indicate that the search for an efficient help for chronic pain patients will not be easy. However, whatever the amount of work needed, the result seems undoubtedly rewarding.

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