

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

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ABSTRACT

Background and objective: The aim of this study was to compare the effects of different paradoxical sleep deprivation methods on the pain threshold in rats submitted to inflammatory and neuropathic pain models. We also investigated whether changes in pain threshold could be reverted by sleep recovery period.

Methods: Wistar rats were randomly assigned in arthritis-induced by adjuvant (AIA), chronic constrictive injury (CCI) of sciatic nerve and non-handled control group. Paradoxical sleep deprivation was performed using small or large platforms 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) paradoxical sleep deprivation.

Results and conclusion: 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. Paradoxical sleep deprivation reduced the pain threshold in all groups studied, independently the method used. Sleep recovery did not restore the baseline pain threshold in arthritis-induced animals, but it was restored in CCI group submitted to both paradoxical sleep deprivation methods.

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

INTRODUCTION

Acute and chronic pain are closely associated with sleep disturbances. Pain has been reported to be an important cause of sleeplessness (1) and, conversely, interrupted sleep has often been associated with increased pain (1-8) and a leading cause of insomnia in medical conditions (3,9). Clinical trials using rheumatic and fibromyalgia patients (10,11) and animal studies with experi-

mental models of polyarthritic rats (8,12-17) 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 would lead to different behavioral alterations, such as increasing pain sensitivity. In fact, some studies report the influence of sleep disturbances on pain sensitivity. However, such influence is not completely understood. Ukponmwan et al. (18) report reduction of antinociceptive

property in enkephalinases, morphine and swimming in paradoxical sleep deprived (PSD) rats. Onen et al. (5) described that threshold of vocalization response to pressure nociceptive stimuli in rats is not reduced by PSD, but it is augmented during the recovery period.

Chronically painful conditions are associated with sleep disturbances such as sleep continuity changes, decreased sleep efficiency and fragmentation of sleep pattern (10,13,15-17,19-24). A recent in-depth review by Lautenbacher et al. (21) highlighted the fact that basic mechanisms governing sleep-induced alterations in pain sensitivity are still unknown, and the difficulty in defining the mechanism by which altered sleep affects pain thresholds suggests a fairly complex chain of events. Among these, sleep-deprived animals might have alterations in the opioidergic receptor system, as hypothesized previously (7,25,26).

The reciprocal influence between pain and sleep-deficit 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 feed-back relationship and aggravate their problems. Some studies (2,4,9) 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 worsens sleep again and enhances pain much more, or if there are adaptative processes, are some of the still unanswered issues. Animal models of chronic pain seem suitable to investigate these questions. Among them, the inflammatory chronic pain of the experimental arthritis induced by adjuvant and the neuropathic chronic pain of the sciatic nerve constriction are 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 (light on at 7:00h) and room temperature ($23 \pm 2^\circ\text{C}$). 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

All animal procedures were approved by the University Ethics Committee (Protocol #065/99). The rats were randomly assigned to three groups: Adjuvant-induced arthritis (AIA), Chronic Constrictive injury (CCI) and non-manipulated controls (CTRL).

Adjuvant-induced arthritis

After administration of the anesthetic (140 mg/kg of ketamine, i.p.), arthritis was induced in 40 animals by a s.c. injection of 0.1 ml of Freund adjuvant (complete fraction of denatured Mycobac-

terium 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 (27).

Study design

The experiment was performed throughout a 9-day period: baseline in dry environment (day 1 and 2), paradoxical sleep deprivation (day 3, 4, 5 and 6) and recovery in dry environment (day 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), the pain threshold was measured and the animals were placed in the tank or remained in the home-cages. Daily, during the 4 days of PSD (Tests 3 to 6) and during the 3 days of recovery (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 in placing ten rats for 96 h 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 stage of paradoxical sleep, it falls into the water, due to muscle atonia, and wakes up. Since the large platforms also produce sleep deprivation, a new proposed control group (28), in which animals are placed onto a grid, was used. The grid group was placed on a stainless steel wire grid, which segments spaced 2.5cm from each other. The grid was fixed horizontally and 1cm above the water surface in the deprivation tank. The other (CTRL) group was housed in plastic cages and allowed to sleep normally.

Assessment of nociception

Pain sensitivity to noxious thermal stimuli was assessed between 9:00h and 11:00h. The hot-plate apparatus to test pain threshold consists of a 20-cm diameter metal hot-plate surface set at 50°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 Dunnett as post hoc test. The level of significance was set at $p < 0.05$.

Table 1: Means (\pm SEM) of pain threshold of normal-pain control (CTRL) animals.

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

*Values significantly different from those of the cage group, $p < 0.05$ (two-way ANOVA followed by post hoc Dunnett test). (PSD: paradoxical sleep deprivation; R: rebound).

Table 2: Means (\pm SEM) of pain threshold in arthritic rats (AIA).

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

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

Table 3: Means (\pm SEM) of pain threshold of rats with chronic constrictive injury of the sciatic nerve.

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

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

RESULTS

The effect of experimental pain models on pain threshold

Two-way ANOVA followed by Dunnett test revealed that AIA and CCI differed from cage 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 induced experimental pain models

The CTRL group (non-manipulated animals) showed decreased latency on the hot plate test during the 4 days of PSD and on the first day of rebound using both small and large platforms. The grid group presented also reduction of pain threshold during the PSD period (Table 1).

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

In regard to CCI animals (Table 3), we observed that the exposure to both small and large platforms 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.

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 (8,21,22,29). 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 the baseline pain threshold in arthritic rats, but it did so in CCI group placed on both small and large platforms. Additionally, the second day of rebound was sufficient to restore pain threshold to baseline values in control animals. The grid method proposed as control environmental to PSD induced an increase of pain threshold latency in AIA animals (test 8 and 9) and a decrease in CCI (test 5 and 6) and control (test 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 known that large platform does not deprive sleep as much as the small one (30). The correlation found between the magnitude of PSD promoted by small and large platforms 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. Neurotransmission systems, such as serotonergic and opioidergic pathways have been investigated in respect to the participation of pain and sleep manipulation (1,5,7). Concerning sleep manipulations, an

inverse relationship between brain serotonergic activity and pain has been reported in several animal studies. Therefore, PSD appears to increase the rate of serotonin metabolism in the rat brain (31).

Ukponmwan et al. (18) reported that 96 hours 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 (5). Earlier, Kay (32) 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 (26).

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 (33). The psychological component has the potential to interact with both pain and sleep further complicating the situation. In addition to this etiological link between depression, chronic pain, and sleep disturbances, depressive patients seems to have greater necessity for paradoxical sleep, since they manifest shorter latencies than normal controls do (34), as well as increased paradoxical sleep time (35,36). The demonstration that rats under chronic pain and submitted to PSD may be used to approach this vicious circle seems to be a promising idea. Recently, Andersen, Hoshino and Tufik (37) reported that animal models for chronic neuropathy exhibit reduced sucrose ingestion. Accordingly, this anhedonic condition that constitutes the core manifestation of depressive states does not occur in response to a single episode of total PSD.

Sleep disturbances attributable to pain suggest a bidirectional relationship between sleep disturbance and pain (29,38,39); both may interact in complex ways that ultimately impact biological and behavioral activity (40). It has been proposed that recognition of disturbed or unrefreshing sleep influences the management of painful medical disorders (29). Specifically, our group has investigated this complex association in animal models as well as clinical conditions (7,10,14-17,19,20,24,39). For instance, it has been demonstrated that arthritis induced by Freund's adjuvant injected into rat hind paws causes reduced sleep efficiency, slow wave sleep and paradoxical sleep, as well as increased latency to the first sleep episode and arousal events (14). Previous studies have also shown similar findings in arthritic rats that were characterized by increased wakefulness, decreased slow wave sleep and paradoxical sleep, and increased sleep fragmentation (13).

We have previously reported that a dose of substance P insufficient to depolarize spinal cord motoneurons of mice tested on a hot plate test was still able to impair the sleep pattern (20). The sleep disturbance found in the group injected with substance P is most likely due to its direct action on the sleep-wake system. Collectively, these findings indicate a strong association between the reduction of the pain threshold and sleep disruption during osteoarthritis, where reduction of the pain threshold is probably

responsible for sleep disturbances that in turn cause further lowering of the pain threshold (8).

It has been proposed that fragmentation of the sleep pattern may promote sleep deprivation (26), which, in turn, increases behavioral responses to mechanical (14), thermal (7), and painful electrical stimuli (5). Sleep deprivation might affect sleep homeostasis and may play an important role in nociception. Indeed, Nascimento et al. (7) showed that the threshold of paw withdrawal after thermal noxious stimulation was reduced by 96 h of PSD and that this effect persisted after 24 hours of sleep recovery. Collectively, our data suggest that chronic pain models may present a valuable framework for the study of the reciprocal influences between non-efficient sleep and pain.

Finally, one may consider 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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