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Effects of Total Sleep Deprivation on Mouse Cytokine Levels

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Cytokines · Total sleep deprivation · Mice

Abstract
Objective: Cytokines play an integral role in sleep/wake regulation. The objective of this study was to identify how total sleep deprivation affects cytokine levels. ***Methods:*** Male C57BL/6 mice were subjected to 48 h of total sleep deprivation produced by brief rotation of activity wheels or 3 different controls: home cage, a sedentary wheel, or forced activity. In addition, the serum levels of cytokines were analyzed using a mouse magnetic bead-based multiplex immunoassay. ***Results:*** The concentrations of some cytokines (fibroblast growth factor-basic [FGF-basic], leukemia inhibitory factor [LIF], and monokine induced by interferon-γ [MIG]) decreased significantly after total sleep deprivation. However, other cytokines (macrophage colony-stimulating factor, macrophage inflammatory protein-2, platelet-derived growth factor-bb, vascular endothelial growth factor) did not show any significant difference. Serum corticosterone levels did not differ significantly among the groups. ***Conclusion:*** The biochemical mechanisms responsible for sleep regulation are very complex. These results suggest the involvement of 3 cytokines (FGF-basic, LIF, and MIG) in sleep/wake regulation.

Introduction

Sleep and sleep pathologies are important for our quality of life. In addition to its effects on cognitive function, a lack of sleep can act as a risk factor for the development of chronic disease, notably cardiovascular and metabolic chronic diseases [1]. A number of studies by several groups have shown an intimate interrelationship between alterations in inflammatory-type cytokine levels and sleep [2]. Interleukin-1β (IL-1β) was first implicated in sleep regulation approximately 30 years ago based on the finding that it has the capacity to enhance non-rapid-eye-movement sleep [3]. Since this study was conducted, the list of these cytokines, including IL-1, IL-6, and tumor necrosis factor (TNF), is quite impressive [4–6].

Studies have suggested that sleep loss promotes exaggerated inflammatory responses in the brain and periphery [7–9]. Rapid-eye-movement sleep deprivation elevated levels of proinflammatory cytokines (IL-1α, IL-1β, IL-6, IL-12, IL-17A, and TNF-α), while the plasma level of the anti-inflammatory cytokine IL-10 did not change, and the level of interferon (IFN)-γ decreased significantly [6, 10]. Human studies have indicated that the effects of sleep loss on proinflammatory gene expression occur via the activation of nuclear factor (NF)-κB,

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the key transcription control pathway in the inflammatory signaling cascade. Additionally, Toll-like receptors (TLRs), particularly TLR-4, play a role in the activation of NF-κB [11–13]. These cytokines not only participate in functions related to the immune system, but also in complex functions of the central nervous system such as cognition [14]. Esumi et al. [15] demonstrated the involvement of inflammatory mediators in the modulation of memory deficits after sleep deprivation. However, physical exercise before acute total sleep deprivation (TSD) prevents proinflammatory responses in the rat hippocampus [13].

The inflammatory response is driven by a complex network of mediators and signaling pathways. In the present study, TSD was induced in mice using the rotating drum method to evaluate whether the cytokine profile would be affected by TSD. The following cytokines were examined: fibroblast growth factor-basic (FGF-basic), leukemia inhibitory factor (LIF), macrophage colony-stimulating factor (M-CSF), monokine induced by IFN-γ (MIG), macrophage inflammatory protein-2 (MIP-2), platelet-derived growth factor-bb (PDGF-bb), and vascular endothelial growth factor (VEGF). The aim of the study was to investigate the involvement of new inflammatory markers in modulating sleep-wake behavior.

Methods

Mice

Male C57BL/6 mice were purchased from Vital River Laboratories (Beijing, China). The animals arrived in the laboratory at least 2 weeks before the experiments. The mice were singly housed and allowed food and water ad libitum. All mice were used at 8–10 weeks of age, and all were trained to a 12-h light/dark cycle (from 07:00 to 19:00). All experiments were performed using mice held at an ambient temperature of 21°C, with animals sacrificed between 9:00 and 10:00 to control for circadian rhythms in cytokine levels. All animal use procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences (Permit No. IOZ11012). All measures were taken to minimize the number of animals used and to minimize suffering.

Total Sleep Deprivation

The in-house-built sleep deprivation device consisted of a rotating drum. The drums were large, motorized, stainless-steel activity wheels with 22-cm diameters and internal wheel widths of 18 cm. The large size of the wheels allowed the mice to move freely. The running wheels were driven by a computer-controlled motor mediated by a drive belt. The front and rear panels consisted of Plexiglas. At the front panel, a water bottle and a feeding rack were mounted. The mice remained singly housed and had free access to food and water while in the wheel. The lens of an infrared sensitive video camera was placed 20 cm ahead of the wheel. Video monitoring continued throughout the deprivation period.

Forty-eight-hour TSD (from 09:00 to 09:00) was achieved by slowly rotating the wheels at a constant speed (0.3 m/min) [16–18]. Three controls were used in the experiment. The first control (sedentary control) was mice that rested on the sedentary wheels. To control for the nonspecific effects of the activity wheel (e.g., mild forced locomotion), a forced activity control group was used. Animals of the forced activity group were placed in the same drums as the ones that were used for TSD. However, these wheels rotated at double speed (0.6 m/min) during the night (12 h), which is their circadian activity phase. With this protocol, the mice had to walk at a higher intensity and had more time to sleep (12 h of rest per day). The third control group (home cage control) remained in their home cages in the same room where the TSD procedure took place.

Before starting the experiments, the mice were habituated to the experimental apparatus by placing them in the wheel. The mice remained singly housed throughout the sleep deprivation experiments in the wheels.

Serum Multiplex Cytokine Array

The serum levels of 7 cytokines were evaluated in serum samples using magnetic bead-based multiplex immunoassays (Bio-Plex; Bio-Rad Laboratories, USA) following the manufacturer's instructions. The blood was prepared according to the manufacturer's instructions. Briefly, whole blood was collected and allowed to clot by leaving it undisturbed at room temperature for 30 min. The clot was then removed by centrifuging at 1,000 *g* for 10 min at 4°C and transferred into a clean tube. The sample was then subjected to centrifugation at 10,000 *g* for 10 min at 4°C. The resulting supernatant was designated serum. The samples were maintained at 2–8°C while handling and immediately analyzed, avoiding freeze-thaw cycles because this is detrimental to many serum components.

The assay was performed according to the manufacturer's instructions and data were acquired using a Bio-Plex 200 reader (Bio-Rad Laboratories, USA). The analysis was performed using Bio-Plex array software, which allows the calculation of cytokine concentrations in unknown samples.

Serum Corticosterone Array

To assess the potential influence of stress caused by the sleep deprivation treatment, serum was delivered to the YAD Biotechnology Company (Beijing, China) for the determination of corticosterone (CORT) levels via an enzyme-linked immunosorbent assay.

Statistical Analyses

The results for each variable were compared using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). The standard error of the mean was calculated and expressed. Data from the sedentary controls, the forced activity controls, and TSD mice were analyzed using one-way analysis of variance followed by Tukey's post hoc test for statistical significance. An unpaired *t* test was used to test for differences between the sedentary controls and the home cage controls. Statistical significance was achieved when $p < 0.05$. Samples sizes in each group were 8 animals.

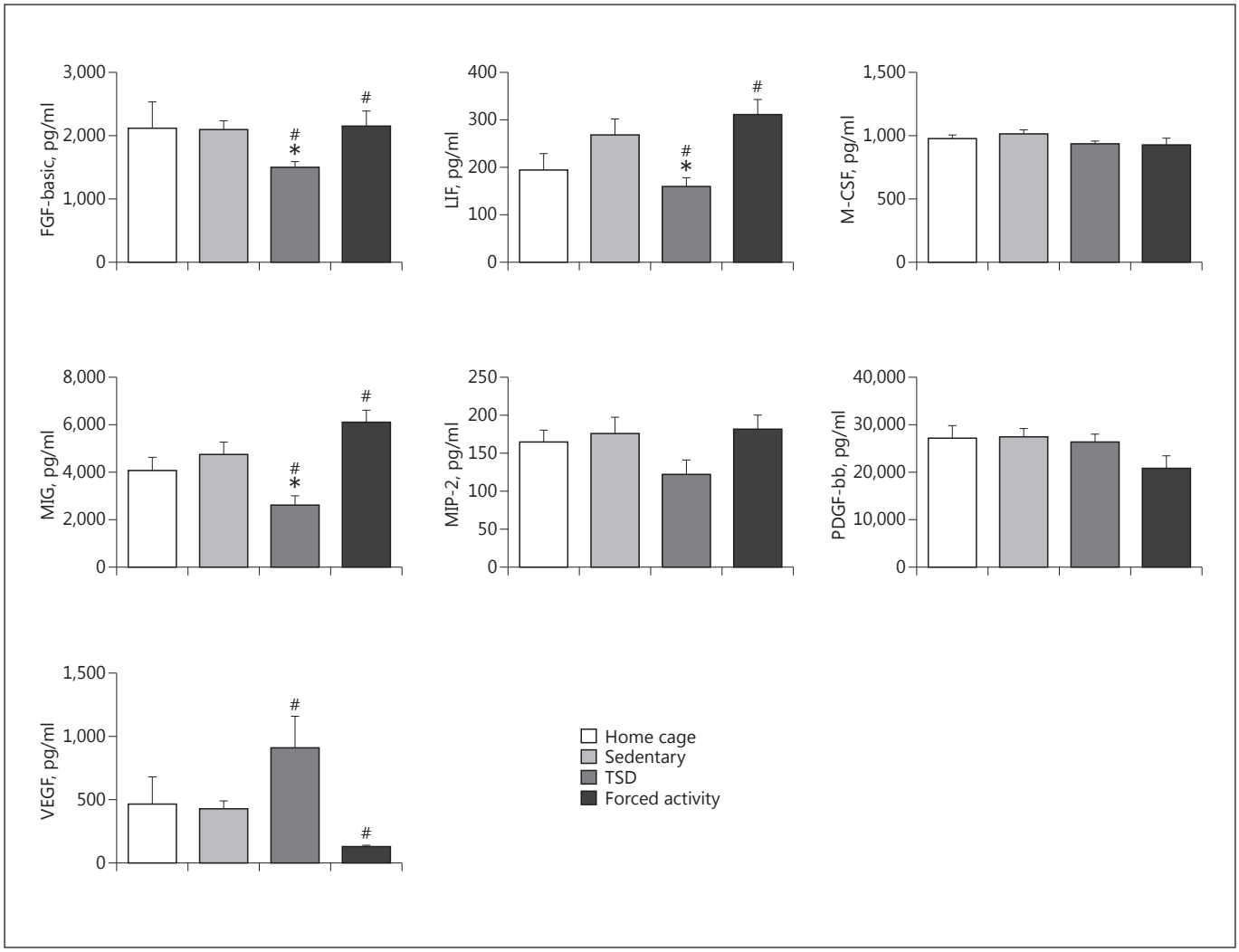

Fig. 1. Serum levels of cytokines in the mice of the home cage control group, the sedentary control group, the forced activity control group, or 48 h of the TSD group. All values are shown as means ± SEM. * Significantly different between the sedentary controls and TSD mice ($p < 0.05$); # significantly different between the forced activity controls and TSD mice ($p < 0.05$).

Results

Serum Level of Cytokines

Using a multiplexed cytokine array, we assayed 7 analytes in each sample from the 4 groups. TSD led to changes in the levels of 7 cytokines (Fig. 1). Overall, the serum levels of 3 cytokines (FGF-basic, LIF, and MIG) in mice deprived of sleep for 48 h were significantly depressed compared with those of the sedentary controls ($p < 0.05$). Other cytokines (M-CSF, MIP-2, PDGF-bb, and VEGF) showed no significant difference between the 2 groups. However, a decreasing trend was found for M-CSF, MIP-2, and PDGF-bb. In contrast, VEGF showed an increasing trend. No significant cytokine concentration changes were found between the forced activity controls and the sedentary controls. Unlike the TSD group, the forced activity control mice displayed a decreasing trend in VEGF concentration and an increasing trend in the FGF-basic, LIF, MIG, and MIP-2 concentrations. Forced activity was found to significantly alter FGF-basic, LIF, MIG, and VEGF levels when compared with the TSD group. The serum levels of 7 cytokines did not differ significantly between the home cage controls and the sedentary controls.

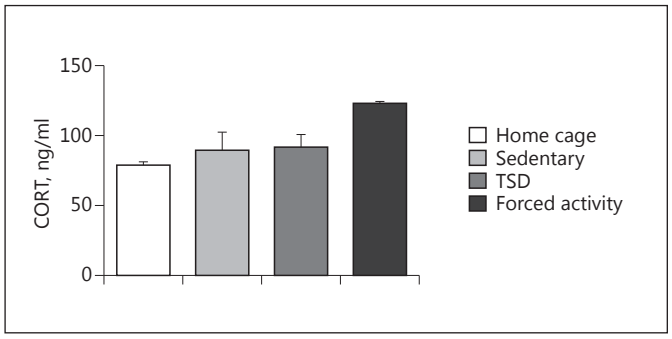

Fig. 2. Serum corticosterone levels immediately after the termination of the sleep deprivation experiment. The bars are plotted as means ± SEM.

Serum Level of CORT

To assess stress levels, we measured the serum CORT levels immediately after the termination of TSD. Levels of CORT were not statistically different among the 4 groups of mice (Fig. 2). In contrast, increased serum CORT was seen in the mice subjected to forced locomotion, but the difference was not significant.

Discussion

In this study, mice were deprived of sleep for 48 h using the rotating drum method and a significant decreasing trend was found for 3 inflammatory mediators (FGF-basic, LIF, and MIG).

There is general consensus that sleep benefits neuronal plasticity, which ultimately supports brain function and cognition [19]. Sleep deprivation impairs hippocampal-dependent learning and memory, which is associated with hippocampal neurogenesis [20]. It has been shown that prolonged sleep deprivation inhibits hippocampal neurogenesis in adult rats [21–23]. FGF-basic is abundantly expressed in the nervous system and was previously shown to support the survival and growth of neurons and neural stem cells in vitro [24]. Intranasal administration of FGF-basic has been shown to promote cerebral neurogenesis [25]. Thus, the decreased level of FGF-basic could represent the negative effects of TSD on hippocampal neurogenesis. FGF-basic regulates the growth and function of vascular cells such as endothelial and smooth muscle cells [26], which might be one of the cellular mechanisms of sleep deprivation-related adverse effects on the cardiovascular system and muscle atrophy [27, 28].

LIF is the most pleiotropic member of the IL-6 family of cytokines. LIF exerts a developmental-dependent effect on a variety of tissues. LIF induces the activation of signaling pathways associated with neuroprotection and regeneration, including the phosphorylation of protein kinase B (Akt) [29, 30]. It has been shown that sleep deprivation induces the preferential atrophy of skeletal muscle tissue through the activities of the Akt/mechanistic target of rapamycin-complex 1 pathway. In the study, sleep deprivation decreased both total Akt and phosphorylated Akt levels [31]. This finding supports our hypothesis that during sleep deprivation, LIF is a modulator of fiber tropism.

The chemokine CXCL9/MIG is a small molecule produced by IFN-γ-stimulated mononuclear cells that acts through its interaction with the CXCR3 receptor, which is found on a variety of cell types. The presence of a decreased level of CXCL9/MIG may be because sleep deprivation decreases the level of IFN-γ [6]. Natural killer cells are one of the major producers of IFN-γ [32], and their activity has been reported to be depressed by sleep disturbances [33, 34].

The stressfulness of sleep deprivation, as implied by increased CORT levels, has been studied before with mixed intervening variables, including exposure to forced locomotion and novel environments. We used forced activity control mice to test whether the effects of TSD might be due to forced activity rather than sleep deprivation per se. In our study, CORT levels were elevated in the forced activity control group, both compared with the sedentary controls and TSD animals, though there were no significant differences. Also, the changes in serum cytokine concentrations of the forced activity controls were different from that of TSD animals. These findings separate the effects of TSD from that of forced activity. To attenuate the effect of novel environments, we established a large rotating drum where mice could move without any hindrance, similar to the home cage. Before the experiment, the mice were given approximately 1 week to acclimatize to the drum [9]. Sedentary control mice exposed to rotating drums exhibited similar serum CORT concentrations relative to mice remaining in their home cage. Our data suggest that highly standardized sleep deprivation methods could possibly overcome such obstacles [35, 36].

Additionally, the emotional stress induced at this speed (0.3 m/min) is benign. Plodding along in a slowly rotating drum appears to be no more emotionally stressful to an animal than an array of varied sensory stimuli such as "gentle handling," all of which frustrate the impulse to sleep [37].

The inflammatory response is driven by a complex network of mediators and signaling pathways. Evaluation of the significance of systemic cytokine modulation is important for understanding the complete mechanism of the immunological changes observed during sleep deprivation. Our current study indicates the possible involvement of 3 cytokines (FGF-basic, LIF, and MIG) in the inflammatory response to TSD. The present study opens up new avenues to evaluate the sleep deprivation-associated modulation of serum protein(s). Due to the interaction between the immune system and the CNS, and particularly the role that cytokines play in that communication, characterization of the impact of cytokines on the electrophysiological properties of neurons will provide a better understanding of sleep-wake behavior. Additional studies could be conducted to determine the effect of cytokine networks on the neuronal circuits associated with the regulation of sleep.

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Disclosure Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1 Faraut B, Boudjeltia KZ, Vanhamme L, Kerkhofs M: Immune, inflammatory and cardiovascular consequences of sleep restriction and recovery. Sleep Med Rev 2012;16:137–149.
2 Krueger JM: The role of cytokines in sleep regulation. Curr Pharm Des 2008;14:3408–3416.
3 Moldofsky H, Lue FA, Eisen J, Keystone E, Gorczynski RM: The relationship of interleukin-1 and immune functions to sleep in humans. Psychosom Med 1986;48:309–318.
4 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;166:1756–1762.
5 Vgontzas AN, Zoumakis E, Bixler EO, Lin HM, Follett H, Kales A, Chrousos GP: Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin Endocrinol Metab 2004;89:2119–2126.
6 Pandey AK, Kar SK: REM sleep deprivation of rats induces acute phase response in liver. Biochem Biophys Res Commun 2011;410:242–246.
7 Simpson N, Dinges DF: Sleep and inflammation. Nutr Rev 2007;65:S244–S252.
8 Del Gallo F, Opp M, RImeri L: The reciprocal link between sleep and immune responses. Arch Ital Biol 2014;152:93–102.
9 Ashley NT, Sams DW, Brown AC, Dumaine JE: Novel environment influences the effect of paradoxical sleep deprivation upon brain and peripheral cytokine gene expression. Neurosci Lett 2016;615:55–59.
10 Yehuda S, Sredni B, Carasso RL, Kenigsbuch-Sredni D: REM sleep deprivation in rats results in inflammation and interleukin-17 elevation. J Interferon Cytokine Res 2009;29:393–398.
11 Irwin MR, Witarama T, Caudill M, Olmstead R, Breen EC: Sleep loss activates cellular inflammation and signal transducer and activator of transcription (STAT) family proteins in humans. Brain Behav Immun 2015;47:86–92.
12 Irwin MR, Wang M, Ribeiro D, Cho HJ, Olmstead R, Breen EC, Martinez-Maza O, Cole S: Sleep loss activates cellular inflammatory signaling. Biol Psychiatry 2008;64:538–540.
13 Chennaoui M, Gomez-Merino D, Drogou C, Geoffroy H, Dispersyn G, Langrume C, Ciret S, Gallopin T, Sauvet F: Effects of exercise on brain and peripheral inflammatory biomarkers induced by total sleep deprivation in rats. J Inflamm (Lond) 2015;12:56.
14 Maier SF, Watkins LR: Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol Rev 1998;105:83–107.
15 Esumi LA, Palma BD, Gomes VL, Tufik S, Hipolide DC: Inflammatory markers are associated with inhibitory avoidance memory deficit induced by sleep deprivation in rats. Behav Brain Res 2011;221:7–12.
16 Campbell IG, Guinan MJ, Horowitz JM: Sleep deprivation impairs long-term potentiation in rat hippocampal slices. J Neurophysiol 2002;88:1073–1076.
17 Roman V, Hagewoud R, Luiten PG, Meerlo P: Differential effects of chronic partial sleep deprivation and stress on serotonin-1A and muscarinic acetylcholine receptor sensitivity. J Sleep Res 2006;15:386–394.
18 McGuire M, Tartar JL, Cao Y, McCarley RW, White DP, Strecker RE, Ling L: Sleep fragmentation impairs ventilatory long-term facilitation via adenosine A1 receptors. J Physiol 2008;586:5215–5229.
19 Kreutzmann JC, Havekes R, Abel T, Meerlo P: Sleep deprivation and hippocampal vulnerability: changes in neuronal plasticity, neurogenesis and cognitive function. Neuroscience 2015;309:173–190.
20 Hairston IS, Little MT, Scanlon MD, Barakat MT, Palmer TD, Sapolsky RM, Heller HC: Sleep restriction suppresses neurogenesis induced by hippocampus-dependent learning. J Neurophysiol 2005;94:4224–4233.
21 Mueller AD, Pollock MS, Lieblich SE, Epp JR, Galea LA, Mistlberger RE: Sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. Am J Physiol Regul Integr Comp Physiol 2008;294:R1693–R1703.
22 Guzman-Marin R, Suntsova N, Stewart DR, Gong H, Szymusiak R, McGinty D: Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. J Physiol 2003;549:563–571.
23 Zagaar M, Alhaider I, Dao A, Levine A, Alkarawi A, Alzubaidy M, Alkadhi K: The beneficial effects of regular exercise on cognition in REM sleep deprivation: behavioral, electrophysiological and molecular evidence. Neurobiol Dis 2012;45:1153–1162.
24 Chen B, He J, Yang H, Zhang Q, Zhang L, Zhang X, Xie E, Liu C, Zhang R, Wang Y, Huang L, Hao D: Repair of spinal cord injury by implantation of bFGF-incorporated HEMA-MOETACL hydrogel in rats. Sci Rep 2015;5:9017.
25 Jin K, Xie L, Childs J, Sun Y, Mao XO, Logvinova A, Greenberg DA: Cerebral neurogenesis is induced by intranasal administration of growth factors. Ann Neurol 2003;53:405–409.
26 Nugent M, AIozzo RV: Fibroblast growth factor-2. Int J Biochem Cell Biol 2000;32:115–120.

27 van Leeuwen WM, Lehto M, Karisola P, Lindholm H, Luukkonen R, Sallinen M, Harma M, Porkka-Heiskanen T, Alenius H: Sleep restriction increases the risk of developing cardiovascular diseases by augmenting proinflammatory responses through IL-17 and CRP. PLoS One 2009;4:e4589.
28 Dattilo M, Antunes HK, Medeiros A, Monico-Neto M, Souza Hde S, Lee KS, Tufik S, de Mello MT: Paradoxical sleep deprivation induces muscle atrophy. Muscle Nerve 2012;45:431–433.
29 Nicola NA, Babon JJ: Leukemia inhibitory factor (LIF). Cytokine Growth Factor Rev 2015;26:533–544.
30 Liu SC, Tsang NM, Chiang WC, Chang KP, Hsueh C, Liang Y, Juang JL, Chow KP, Chang YS: Leukemia inhibitory factor promotes nasopharyngeal carcinoma progression and radioresistance. J Clin Invest 2013;123:5269–5283.
31 de Sa Souza H, Antunes HK, Dattilo M, Lee KS, Monico-Neto M, de Campos Giampa SQ, Phillips SM, Tufik S, de Mello MT: Leucine supplementation is anti-atrophic during paradoxical sleep deprivation in rats. Amino Acids 2016;48:949–957.
32 Feng CG, Kaviratne M, Rothfuchs AG, Cheever A, Hieny S, Young HA, Wynn TA, Sher A: NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with Mycobacterium tuberculosis. J Immunol 2006;177:7086–7093.
33 Fondell E, Axelsson J, Franck K, Ploner A, Lekander M, Bälter K, Gaines H: Short natural sleep is associated with higher T cell and lower NK cell activities. Brain Behav Immun 2011;25:1367–1375.
34 De Lorenzo BHP, de Oliveira Marchioro L, Greco CR, Suchecki D: Sleep-deprivation reduces NK cell number and function mediated by β-adrenergic signalling. Psychoneuroendocrinology 2015;57:134–143.
35 Fenzl T, Romanowski CP, Flachskamm C, Honsberg K, Boll E, Hoehne A, Kimura M: Fully automated sleep deprivation in mice as a tool in sleep research. J Neurosci Methods 2007;166:229–235.
36 Suchecki D, Tufik S: Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat. Physiol Behav 2000;68:309–316.
37 Rechtschaffen A, Bergmann BM, Gilliland MA, Bauer K: Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat. Sleep 1999;22:11–31.