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Original Article

Effects of MAPK/ERK pathway on learning and memory in sleep deprivation rats

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Abstract: Objective: This study aims to analyze the relationship between changes in MAPK/ERK signaling pathway and learning/memory in sleep deprivation rats, and explore the mechanism of memory impairment in those rats. Methods: Sixty male rats were randomly divided into two groups: control group and sleep deprivation group. The sleep deprivation model was prepared using a multi-platform water environment method. The Morris water maze was employed to observe the changes on spatial navigation and spatial search abilities of rats in control group and sleep deprivation group, respectively. Meanwhile, western blot was used to detect MAPK/ERK signaling pathway-related protein changes. Results: With the increasing number of training, the frequency of the search platform was improved gradually. The search time and exploration distance also were decreased in both groups. The escape latency of rats in the sleep deprivation group was significantly longer than that in the control group ($P<0.05$), and the exploration distance was significantly increased than that in the control group ($P<0.05$). Comparing the ability of space exploration between the two groups, the percentages of exploration time in the sleep deprivation group on the original platform (1, 2, and 4 quadrants) were significantly lower than those in the control group (all $P<0.05$). The total number of crossed times on the platform in the control group (12.51±2.14) was significantly higher than that in the sleep deprivation group (6.03±3.07, $P<0.001$). Western blot results showed that the expression levels of Ras, Raf-1, MEK1/2, ERK1/2, p-ERK1/2, and p-ERK/ERK in the control group were significantly higher than those in the sleep deprivation group. B-Raf expression in the control group was significantly lower than that in the sleep deprivation group. Conclusion: Sleep deprivation can affect learning and memory in rats, and its mechanism may be related to the MAPK/ERK signaling pathway.

Keywords: Sleep deprivation, MAPK/ERK pathway, learning, memory

Introduction

Sleep deprivation (SD) usually refers to the state or process of abnormal sleep rhythm or severe lack of sleep (less than 4 hours per day) [1]. Sleep deprivation can easily lead to the decreased levels of behavior, lack of concentration, memory loss, and decreased learning ability, which can result in serious death [2]. In recent years, studies have found that sleep deprivation can affect body health to varying degrees, but research on the mechanism of the effect of sleep deprivation on learning and memory ability is still limited [3, 4].

The mitogen-activated protein kinase (MAPK) superfamily is a kind of highly evolved enzyme, that is able to associate with intracellular and extracellular information pathway. The main members of the MAPK family, ERK1/2 and MEK, produce effects when they receive external stimuli. Then these proteins can transduce extracellular signals into the nucleus and complete the regulation of cell functions. MAPK/ERK signaling pathway is one of the most widely studied signal transduction pathways. It participates in various physiological and pathological processes such as cell proliferation, growth, and differentiation [5].

At present, long-term potentiation (LTP) is thought to be involved in the process of learning and memory in the brain. LTP can be induced by activating N-methyl-D-aspartate (NMDA) [6, 7]. The previous study has shown that blocking the MAPK/ERK signaling pathway can

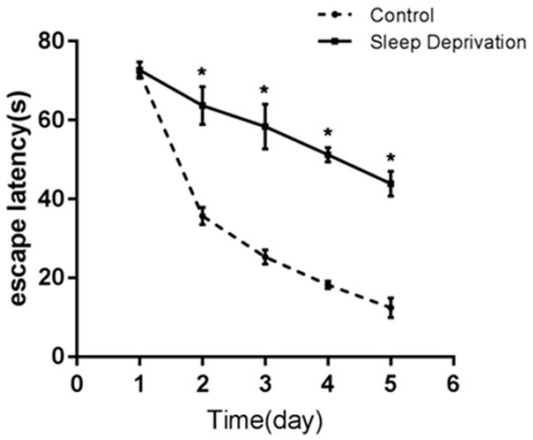

Figure 1. Comparison of escape latency between two groups of rats. *P<0.05.

inhibit the generation of LTP, thereby affect the brain's ability to learn and memory [8]. Therefore, we believe that MAPK/ERK plays an important role in brain learning and memory.

In this study, a sleep deprivation rat model was established to observe the behavioral changes in the Morris water maze after sleep deprivation in rats. Simultaneously, the changes of the related protein in the hippocampus of the rats were examined to investigate the mechanism of the effects in sleep deprivation on learning and memory abilities in rats.

Materials and methods

Animal information

This study was approved by Experimental Animal Ethics Committee of Zhucheng People's Hospital. Adult SD male rats (198-274 g, purchased from Kay Science and Technology, China) were selected. Adaptive feeding was used during one week, and the rats could eat and drink freely. Then the rats were randomly divided into the control group and the sleep deprivation group using a random sampling method. Each group contained 30 animals. The control group was treated with a platform control system (no rotation of the breeding platform). The rats in the sleep deprivation group were subjected to 6 days of sleep deprivation based on the requirements.

Sleep deprivation rat model preparation

Using a sleep deprivation device (SA107, SANS Biological Technology, China), a rat sleep deprivation model was prepared according to the instrument operating instructions, and 6 days of continuous sleep deprivation was performed as follows. The experimental rats were placed in a sleep deprivation apparatus to provide adequate food and water. The platform was set to rotate forward and backward, and the running speed was 100 rpm/min. When the rats entered into rapid eye movement (REM) sleep and the whole body muscle tension decreased, the standing platform of the rats moved randomly. Thus, the rats could not enter into out-of-phase sleep at all times. The phenomenon of unresponsiveness, apathy, and decreased alertness in rats indicates that the rat model of sleep deprivation was successfully prepared [9].

Morris water maze experiment

The Morris water maze tracking system (WMT-100S, Taimeng, China) was used for positioning navigation and space exploration experiments [10]. Positioning navigation experiment was performed as follows. The experimental training period was 4 days, which was divided into morning and afternoon periods. In each period, the rats were trained 4 times. The platform quadrant was randomly selected as the water entry point to record the time that the rats reached the platform from the water (the escape latency). If the rats stayed in the water for more than 90 s, they were guided to the platform artificially, and the escape latency was recorded as 90 s.

After the training, the rats in the control group were reared on the platform, and the rats in the sleep deprivation group were subjected to sleep deprivation for 1, 2, 3, 4 and 5 days. The positioning navigation experiments were performed daily to record the escape latency of the control group and the sleep deprivation group. Space exploration experiment was performed as follows. After withdrawing the sleep deprivation platform (6 days), the original quadrant of the platform was recorded as the target area. The number of times that the rat crossed the original platform in 120 s was measured. The ratio of the time in the original platform' quadrant to the total time was also recorded.

Western blot determination

The expression of key proteins in the MAPK/ERK pathway in the rat hippocampus was

Table 1. Comparing the exploration distances between the control group and the sleep deprivation group

Day	Control group (cm)	Sleep Deprivation group (cm)	t	P
1	364.27±290.58	382.76±213.39	-0.281	0.780
2	286.36±74.93	374.83±190.74	-2.365	0.021
3	237.82±82.73	358.23±109.09	-4.817	<0.001
4	176.53±23.96	281.61±37.85	-12.848	<0.001
5	125.73±31.65	193.06±42.12	-7.000	<0.001

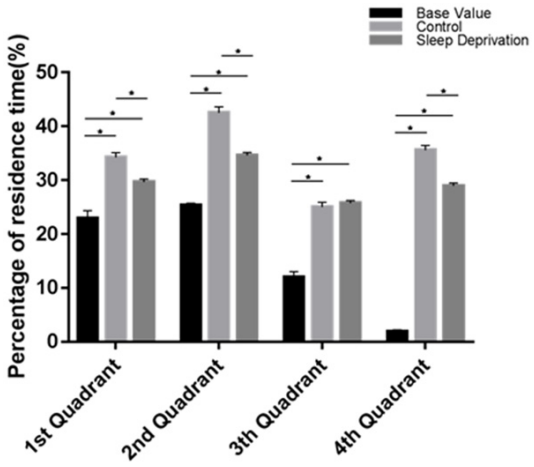

Figure 2. Comparison of the percentages of exploration time in the original platform's quadrants between two groups of rats. *P<0.05.

detected [11]. After the completion of the space exploration experiment in rats, they were sacrificed, and the whole brain were quickly extracted. The bilateral hippocampal tissues were peeled off on ice, frozen in liquid nitrogen and ground. The total protein was extracted with RIPA lysate (P0013D, Beyotime Biotechnology, China).

Protein was quantified according to the procedures of BSA protein quantitation kit (PA115-01, Tiangen, China). SDS-PAGE gel electrophoresis was used for 80 min. Then the protein was transferred to membrane. It was blocked with 5% skim milk powder at room temperature for 1 h, and incubated with primary antibodies (anti-Ras: SAB4301113, Merck; anti-B-Raf: 7H30L21, Invitrogen; anti-Raf-1: ab1735-39, Abcam; anti-MEK1/2: ab178876, Abcam; anti-ERK1/2: ab17942, Abcam; anti-p-ERK1/2, 4377, CST; anti-β-actin: ab8226, Abcam) overnight at 4°C. After washing with 1* TBST on the next day (3 times for 5 min each time), the corresponding resistant secondary antibodies (goat anti-rabbit H&L antibody ab6721, Abcam; goat anti-mouse H&L antibody: ab6789, Abcam) were added, and incubated for 2 h at room temperature. Then the membrane was washed 3 times for 5 min with 1 × TBST. Finally, the membrane was developed (P0018, Beyotime Biotechnology, China) and photographed. The image J software was performed to evaluate the protein by gray-scale analysis.

Statistical analysis

SPSS 21.0 software was used for statistical analysis. The measurement data was expressed as mean ± standard deviation ($\bar{x}$ ± sd). The measurement data with normal distribution was conducted with t test. The comparison of incubation period and exploration distance between the control group and the sleep deprived rats were performed using multivariate analysis of variance. The difference in the relative gray value between the two groups was determined by t test and expressed by t. P<0.05 indicates statistically significant difference.

Results

The effect of sleep deprivation on positioning navigation

In the positioning navigation experiment, the escape latency of rats in the sleep deprivation group was significantly extended with the prolonged sleep deprivation time, and the exploration distance was also longer. Moreover, the sleep latency of rats in the sleep deprivation group was longer than that in the control group on the 2nd, 3rd, 4th, and 5th day, respectively (all P<0.05, **Figure 1**). After analyzing and comparing the exploration distances between the two groups of rats, we found that as the time of sleep deprivation was increased, the exploration distances by the rats were decreased. However, the exploration distances in the sleep deprivation group were longer than that in the control group (all P<0.05, **Table 1**).

The effect of sleep deprivation on space exploration

In the space exploration experiment, the percentage of exploration time in the original plat-

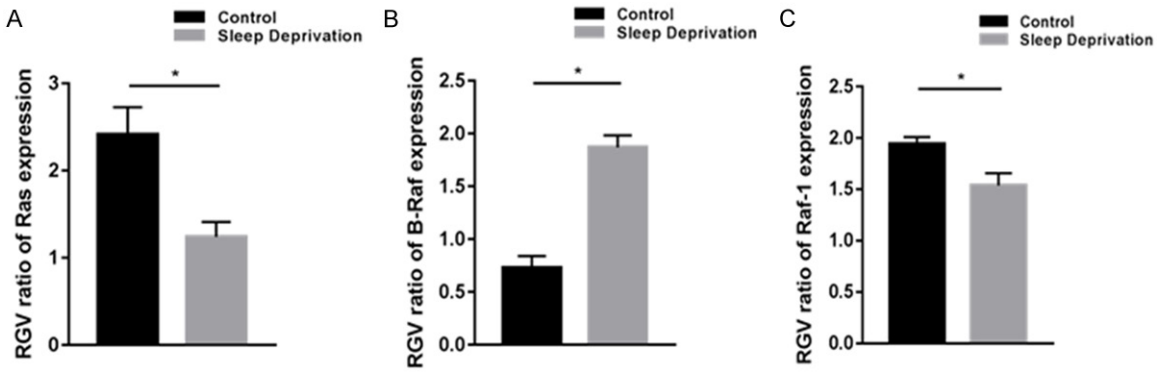

Figure 3. Ras, Raf-1, and B-Raf protein expression in hippocampus of rat. A: Expression of Ras protein in two groups; B: Expression of B-Raf protein in two groups; C: Expression of Raf-1 protein in two groups (n=30, experiment repeated three times). RGV: Relative grey value. *P<0.05.

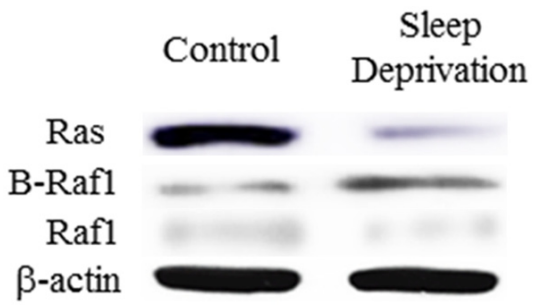

Figure 4. Expression of Ras, Raf-1, B-Raf proteins in hippocampus of rat on western blot.

form quadrant of the rats in the control group and the sleep deprivation group were higher than the baseline value before the training. The percentages of exploration time of the rats in the sleep deprivation group on the original platform 1, 2, and 4 quadrants were significantly lower than that in the control group (all P<0.05). There was no significant difference in the percentage of exploration time between the two groups in the third quadrant (**Figure 2**). The total number of crossing platforms in the control group (12.51±2.14) was significantly higher than that in the sleep deprivation group (6.03±3.07). The difference was statistically significant (t=9.484, P<0.001).

Ras, Raf-1, B-Raf protein expression in rat hippocampus

The Ras, Raf-1, and B-Raf proteins in the hippocampus of rats in the control and sleep deprivation groups were detected by western blot. The results showed that the expression levels of Ras (t=5.667, P=0.005) and Raf-1 (t=12.41, P<0.001) in the hippocampus of rats in the sleep deprivation group were significantly lower than the control group. Moreover, the expression level of B-Raf protein was significantly increased (t=5.024, P=0.007, **Figures 3**, **4**).

Expression of MEK1/2, ERK1/2, p-ERK1/2 proteins in rat hippocampus

The protein expressions of MEK1/2, ERK1/2 and p-ERK1/2 in hippocampus of rats were detected by western blot. The results showed that the expression levels of MEK1/2 (t=4.114, P=0.015), ERK1/2 (t=5.364, P=0.006), p-ERK1/2 (t=7.538, P=0.002) and, p-ERK1/2 and ERK1/2 ratio (t=3.888, P=0.018) in the hippocampus of rats in the sleep deprivation group were significantly lower than the control group (**Figures 5**, **6**).

Discussion

Sleep deprivation usually refers to a state formed by the process of the surrounding environment and its own factors leading to incapable or inadequate sleep [12]. At present, people's living rhythm continues to accelerate, and the work pressure is gradually increasing. More and more people are being threatened by lack of sleep, sleep restriction, and long-term potential sleep deprivation state [13]. In recent years, studies on epidemiology, human sleep deprivation tests, and animal sleep deprivation experiments have found that people or animals that were in a state of sleep deprivation for a long time can cause fatigue, irritability, and sub-healthiness. It

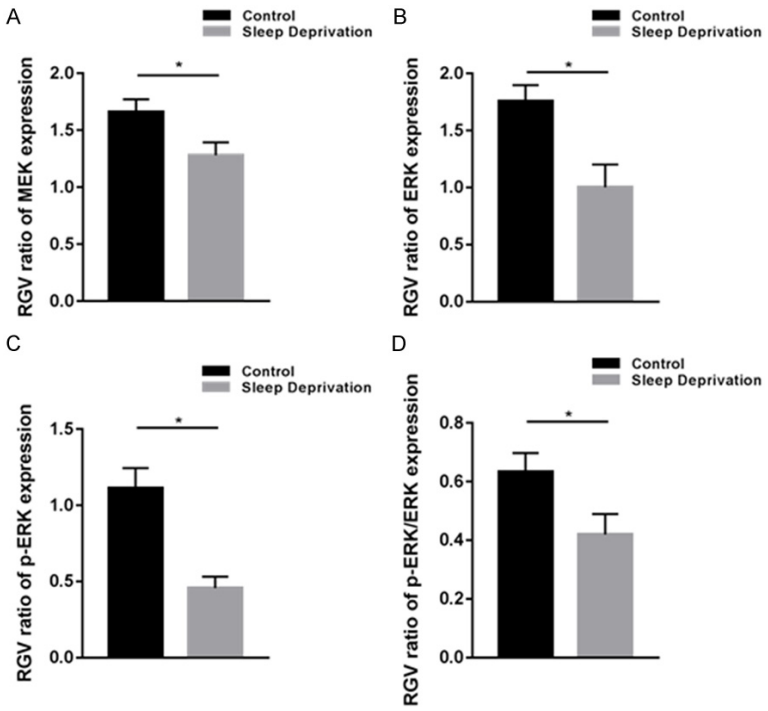

Figure 5. Expression of MEK1/2, ERK1/2, p-ERK1/2 proteins in hippocampus of rat. A: MER protein expression in the two groups of rats; B: ERK protein expression in the two groups; C: p-ERK protein expression in the two groups; D: p-ERK and ERK ratio in the two groups (n=30, experiment repeated three times). RGV: Relative grey value. *P<0.05.

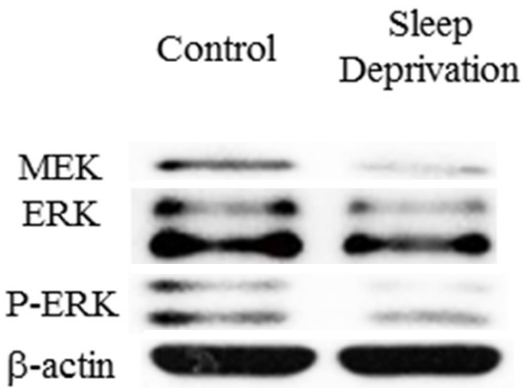

Figure 6. Expression of MEK1/2, ERK1/2, p-ERK1/2 proteins in hippocampus of rat on western blot.

would lead to confusion in thinking, learning and memory, impaired performance and other phenomena [14-18]. The degree of impairment in learning and memory ability of humans and animals is proportional to the duration of sleep deprivation [19]. However, the mechanism of the effects of sleep deprivation on learning and memory is not yet clear.

As an important extracellular signal transduction pathway, MAPK can transduce signals into the nucleus and make cells produce effects. It includes three pathways: ERK, protein-regulated kinase p38, and c-jun end-regulated kinase. MAPK/ERK signaling pathway can activate ERK through multiple pathways in cells. After activation, ERK can participate in learning and memory formation from many aspects [20, 21]. ERK mainly includes two configurations: ERK1 and ERK2. Some studies have found that ERK was associated with LTP [22-24]. When ERK expression was inhibited, it can lead to LTP induction block in hippocampal CA1 region. LTP and learning/memory processes are closely related. Its inhibition of expression would also affect learning and memory [23]. It is believed that MAPK activation of ERK, can play an important role in the process of learning and memory.

As a classic experiment, the Morris water maze was mainly used to analyze the spatial and memory changes of animals [25]. Zhu et al. found that sleep deprivation rats could be studied using Morris water maze test [26]. In that study, the escape latency and exploration distance in sleep deprivation rats were significantly higher than that in the control group. The percentage of search time on the original platform was significantly lower than that of the control group. They confirmed that the spatial memory of rats was significantly reduced after sleep deprivation. This study explored the effect of sleep deprivation on learning and memory in rats through the Morris water maze test. The analysis of rats' navigation and spatial exploration abilities showed that, with the prolonged duration of sleep deprivation, the escape latency of rats in the sleep deprivation group was significantly prolonged than that in the control group. The explored distance was significantly increased than that in the control group. Comparing the ability of space exploration, the percentages of exploration time in the original platform's quadrant of the rats in the control and sleep deprivation group were higher than the baseline value before the training.

The percentages of the rats in the sleep deprivation group on the original platform 1, 2, and 4 exploration time were lower than that in the control group. The total number of crossing the platform was significantly lower than that of the control group. The difference was statistically significant and consistent with the above studies. Therefore, sleep deprivation can lead to a decrease in learning and memory of rats.

Ras, the promoter of the MAPK/ERK pathway, is a GTP-binding protein. Ras is activated by signal transduction receptors and can bind with Raf-1 and B-Raf. Then it will activate their functions [27]. Raf-1 and B-Raf can bind together and activate MEK1/2. Then the activated MEK can bind with ERK1/2. Activated ERK1/2 transmits signals into the nucleus and affects the related transcription factors to achieve cell regulation. Zhang et al. used inhibitors to block the MAPK/ERK signaling pathway in rats. In that study, western blot results showed that decreased levels of ERK phosphorylation can influence learning and memory in rats [28].

This study found that the expression of Ras, Raf-1, MEK1/2, ERK1/2, and p-ERK1/2 proteins in the MAPK/ERK pathway, the ratio of relative expression level of p-ERK1/2 and ERK1/2 in the sleep-deprived rats were significantly lower than that in the control group. These results indicated that the MAPK/ERK signaling pathway was inhibited in the sleep deprivation group. The protein expression level of B-Raf in the sleep deprivation group was significantly increased. This was considered as its widespread distribution. Therefore, we believe that the declination of learning and memory in sleep deprivation rats may be related to inhibition of MAPK/ERK signaling pathway. However, there were still limitations in this study. The sample size was relative small, and we only performed western blot on MAPK/ERK related proteins in this study. Thus, we will examine the expression of MAPK/ERK protein in rat brain tissue by immunohistochemical staining to determine the change.

In conclusion, the effects of sleep deprivation on learning and memory in rats may be related to the changes of expression of key proteins in the MAPK/ERK pathway in the hippocampus.

Disclosure of conflict of interest

None.

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References

[1] Walker MP. The role of sleep in cognition and emotion. Ann N Y Acad Sci 2010; 1156: 168-197.

[2] Whitney P, Hinson JM, Satterfield BC, Grant DA, Honn KA, Van Dongen HPA. Sleep deprivation diminishes attentional control effectiveness and impairs flexible adaptation to changing conditions. Sci Rep 2017; 7: 214-223.

[3] Touitou Y, Reinberg A, Touitou D. Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: health impacts and mechanisms of circadian disruption. Life Sci 2017; 173: 94-106.

[4] Stanojevic C, Simic S, Milutinovic D. Health effects of sleep deprivation on nurses working shifts. Med Pregl 2016; 69: 183-188.

[5] Zhang G, Cheng Y, Zhang Q, Li X, Zhou J, Wang J, Wei L. ATX-LPA axis facilitates estrogen-induced endometrial cancer cell proliferation via MAPK/ERK signaling pathway. Molecular Medicine Reports 2018; 17: 4245-4252.

[6] Yuan LL, Adams JP, Swank M, Sweatt JD, Johnston D. Protein kinase modulation of dendritic K+ channels in hippocampus involves a mitogen-activated protein kinase pathway. J Neurosci 2002; 22: 4860-4868.

[7] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008; 14: 837-842.

[8] Watabe AM, Zaki PA, O'Dell TJ. Coactivation of beta-adrenergic and cholinergic receptors enhances the induction of long-term potentiation and synergistically activates mitogen-activated protein kinase in the hippocampal CA1 region. J Neurosci 2000; 20: 5924-5931.

[9] Gessa GL, Pani L, Fadda P, Fratta W. Sleep deprivation in the rat: an animal model of mania. Eur Neuropsychopharmacol 1995; 5 Suppl: 89-93.

[10] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related

forms of learning and memory. Nat Protoc 2006; 1: 848-858.
[11] Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007; 26: 3291-3310.
[12] Krause AJ, Simon EB, Mander BA, Greer SM, Saletin JM, Goldstein-Piekarski AN, Walker MP. The sleep-deprived human brain. Nat Rev Neurosci 2017; 18: 404-418.
[13] Samatra DPGP, Kesanda IMP, Adnyana IMO, Widyadharma E. The effect of partial sleep deprivation in decrease of cognitive function in resident doctors of Udayana University/Sanglah general hospital. International Journal of Science & Research 2017; 6: 215-218.
[14] Institute of Medicine (US) Committee on Sleep Medicine and Research, Colten HR, Altevogt BM. Sleep disorders and sleep deprivation: an unmet public health problem. Journal of the American Academy of Child & Adolescent Psychiatry 2008; 47: 473-474.
[15] Ben Simon E, Maron-Katz A, Lahav N, Shamir R, Hendler T. Tired and misconnected: a breakdown of brain modularity following sleep deprivation. Hum Brain Mapp 2017; 81: 3300-3314.
[16] Wadhwa M, Kumari P, Chauhan G, Roy K, Alam S, Kishore K, Ray K, Panjwani U. Sleep deprivation induces spatial memory impairment by altered hippocampus neuroinflammatory responses and glial cells activation in rats. J Neuroimmunol 2017; 312: 38-48.
[17] Qiu H, Zhong R, Liu H, Zhang F, Li S, Le W. Chronic sleep deprivation exacerbates learning-memory disability and Alzheimer's disease-like pathologies in AβPP(swe)/PS1(ΔE9) mice. J Alzheimers Dis 2016; 50: 669-685.
[18] Chaput JP, Dutil C. Lack of sleep as a contributor to obesity in adolescents: impacts on eating and activity behaviors. Int J Behav Nutr Phys Act 2016; 13: 103.
[19] Hagewoud R, Whitcomb SN, Heeringa AN, Havekes R, Koolhaas JM, Meerlo P. A time for learning and a time for sleep: the effect of sleep deprivation on contextual fear conditioning at different times of the day. Sleep 2010; 33: 1315-1322.
[20] Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, Ludlam MJ, Stokoe D, Gloor SL, Vigers G, Morales T, Aliagas I, Liu B, Sideris S, Hoeflich KP, Jaiswal BS, Seshagiri S, Koeppen H, Belvin M, Friedman LS, Malek S. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature 2010; 464: 431-435.
[21] Kolch W. Coordinating ERK/MAPK signalling through scaffolds and inhibitors. Nat Rev Mol Cell Biol 2005; 6: 827-837.
[22] Visochek L, Grigoryan G, Kalal A, Milshtein-Parush H, Gazit N, Slutsky I, Yeheskel A, Shainberg A, Castiel A, Seger R, Langelier MF, Dantzer F, Pascal JM, Segal M, Cohen-Armon M. A PARP1-ERK2 synergism is required for the induction of LTP. Sci Rep 2016; 6: 24950.
[23] Chen X, Wang Y, Xu F, Wei X, Zhang J, Wang C, Wei H, Xu S, Yan P, Zhou W, Mody I, Xu X, Wang Q. The rapid effect of bisphenol-A on long-term potentiation in hippocampus involves estrogen receptors and ERK activation. Neural Plast 2017; 2017: 5196958.
[24] Tanasic S, Mattusch C, Wagner EM, Eder M, Rupprecht R, Rammes G, Di Benedetto B. Desipramine targets astrocytes to attenuate synaptic plasticity via modulation of the ephrin A3/EphA4 signaling. Neuropharmacology 2016; 105: 154-163.
[25] D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 2001; 36: 60-90.
[26] Zhu C, Yao X, Zhang W, Song Y, Hou Y. Progressive paradoxical sleep deprivation impairs partial memory following learning tasks in rats. Neural Regen Res 2008; 3: 598-603.
[27] Athuluri-Divakar SK, Vasquez-Del Carpio R, Dutta K, Baker SJ, Cosenza SC, Basu I, Gupta YK, Reddy MV, Ueno L, Hart JR, Vogt PK, Mulholland D, Guha C, Aggarwal AK, Reddy EP. A small molecule RAS-mimetic disrupts RAS association with effector proteins to block signaling. Cell 2016; 165: 643-655.
[28] Zhang JJ, Okutani F, Inoue S, Kaba H. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathway leading to cyclic AMP response element-binding protein phosphorylation is required for the long-term facilitation process of aversive olfactory learning in young rats. Neuroscience 2003; 121: 9-16.