Int J Clin Exp Med 2016;9(2):2383-2390
www.ijcem.com /ISSN:1940-5901/IJCEM0017244

Original Article

Brain-specific expression of cellular prion protein in memory impairment induced by sleep-deprivation in rats

Pujun Zhao[1*], Jun Sun[1*], Guang Sun[2*], Wei Dou[3]

[1]Division of Clinical Pharmacology, General Hospital of Jinan Military Command, Jinan 250031, China; [2]Division of ICU, NO. 1 People's Hospital of Zibo City, Zibo 255200, China; [3]Division of Neurology, 456 Hospital of PLA, Jinan 250031, China. [*]Equal contributors.

Received October 2, 2015; Accepted December 10, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Objective: We designed this study to investigate the mechanism of cellular prion protein (PrP^C) in memory impairment induced by sleep-deprivation. Methods: Rats were deprived of sleep for 72 h using the modified multiple platform method. The Morris water maze task was used to assess hippocampal-dependent spatial memory. After sleep-deprivation, the rats were sacrificed and their brain tissue was analyzed for PrP^C and cAMP response element binding protein (CREB) expression by western blotting and immunohistochemistry. Results: After 72 h sleep-deprivation, it was observed hippocampal-dependent spatial memory impairment, down-regulations of PrP^C and p-CREB (selectively in the hippocampus) in rats. Conclusions: These findings suggest that the reduced PrP^C level in the hippocampus contributes to sleep-deprivation induced hippocampal-dependent memory impairment; and CREB signaling pathway involves in this process. In view of PrP^C-CREB system involves in sleep-deprivation induced memory impairment, it may be a potential target for future treatment.

Keywords: Sleep, deprivation, memory, prions, CREB, rat

Introduction

It is well known that sleep-deprivation can lead to serious physiological disorders and even death [1]. Although previous data indicate that sleep-deprivation impairs learning and memory [2, 3], the mechanistic details of this process still remain unclear. A glycosylphosphatidylinositol-anchored membrane protein-cellular prion protein (PrP^C) is involved in memory, sleep, inflammatory reactions, cellular proliferation and cellular differentiation [4]. Among these functions, the role of PrP^C in memory and sleep is intriguing. Research has shown that PrP^C-null mice have defects in hippocampal-dependent spatial learning, which can be reversed by PrP^C being selectively re-expressed in neurons [5]. In addition, PrP^C-null mice exhibit alterations in their circadian rhythms and sleep patterns [6], which indicates that PrP^C might be involved in sleep regulation. All above data suggest that PrP^C is involved in both memory and sleep function in mice. The cAMP response element binding protein (CREB) is a transcription factor that is involved in many physiological processes, including learning and memory. Previous studies indicate that p-CREB levels were increased in the hippocampus following learning, and down-regulated after sleep-deprivation [7, 8], which suggest that CREB phosphorylation may play important role in the cognitive impairment induced by sleep-deprivation.

Despite efforts having been made, it is still unclear the way that PrP^C and CREB phosphorylation involved in sleep-deprivation induced memory impairment. This study was designed to explore the details of cerebral PrP^C function in. We hope that will bring greater understanding to mechanism of memory impairment induced by sleep-deprivation.

Materials and methods

Ethics statement

This protocol followed the national guidelines for the care and use of experimental animals

(China). And protocol were reviewed and approved by the experimental animal ethics committee of the Second Military Medical University (Approval Number: AE2013-0146). The animals were maintained under standard conditions, which included free access to food and water. Clean cages and fresh water were provided twice a week.

Chemicals

The chemicals used in this study included: rabbit monoclonal antibody (EP1802Y) to the prion protein PrP (Abcam, Cambridge, England, UK), CREB (48H2) rabbit monoclonal antibody, phospho-CREB (Ser133) (87G3) rabbit monoclonal antibody (Cell Signaling, Boston, Massachusetts, USA), mouse monoclonal antibody to β-actin, mouse monoclonal antibody to GAPDH (Kangcheng, Shanghai, China), goat anti-rabbit IgG horseradish peroxidase (HRP)-linked antibody, donkey anti-rabbit IgG Cy3-linked antibody (Jackson, Lancaster, Pennsylvania, USA), DMEM/F12, fetal bovine serum, and B27 (Invitrogen, Camarillo, California, USA). Water was distilled and purified by Milli-Q water purification system (Millipore, Bedford, Massachusetts, USA). All other chemicals were of analytical grade.

Rats and treatment

Adult male Sprague-Dawley rats (weight 220-250 g) and new born rats were used. Adult male rats were randomly divided into 3 groups: cage control (CC) group, tank control (TC) group, and sleep-deprivation (SD) group. All rats were allowed to acclimate for 2 weeks before the experiment; rats of the TC and SD groups were allowed to acclimate on platforms for 1 h per day for 1 week. The rats were raised under an automatic 12 h light/12 h dark cycle (lights on at 08:00) at 24°C, 50-60% humidity. The rats were anesthetized with chloral hydrate immediately after completing the 72 h sleep-deprivation period and the Morris water maze probe test. The new born rats were sacrificed for experiment of hippocampal neurons culture.

Sleep-deprivation

Rats of SD group were deprived of sleep for 72 h using the modified multiple platform method, which has been reported to interfere with both REM (rapid eye movement) sleep and non-REM sleep [9, 10]. When the rats placed on the small platforms reached the REM sleep phase, they would be woken up by the touch of water due to loss of muscle tone. Rats placed on the large platforms set to be TC group, which enabled us control the potential environmental stress effects. The other rats were maintained in their home cages as normal controls (CC).

Assessment of spatial memory

The Morris water maze task, which is widely used to assess hippocampal-dependent learning and memory, was performed as described previously [11]. Memory retention was evaluated based on the number of times the rats passed through the original site of the platform and the mean value of proximity to the platform. The swimming trail was recorded simultaneously. After the training period, 18 rats (6 per group) were used in a probe test to acquire the baseline spatial memory data. During the probe test, the platform was removed to keep the rats swimming freely for 120 s. After the 72-h sleep-deprivation period was over, the probe test was immediately performed again.

Western blotting

The tissue or cultured cells were collected, homogenized, and centrifuged. The supernatants were collected, mixed with loading buffer, boiled for 5 min, electrophoretically separated on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked with 10% nonfat dry milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 2 h at room temperature and subsequently incubated with PrP (1:10000, Abcam), p-CREB (1:500, Cell Signaling) and CREB (1:1000, Cell Signaling) antibodies in TBST overnight at 4°C. After incubation with HRP-conjugated secondary antibodies (1:10000, Jackson) for 2 h at room temperature, the bands were visualized using an ECL detection kit. The optical density of the bands was analyzed using Image-Pro Plus 6.0 image software.

Immunohistochemistry

A 0.9% NaCl solution and 4% paraformaldehyde in 0.1 M PBS were used to perfuse in rats. The brains were dissected out, immersed in 4% paraformaldehyde in 0.1 M PBS for 4 h and transferred to 20% sucrose in 0.1 M PBS until they sank to the bottom. Frozen sections were used for p-CREB detection by immunofluores-

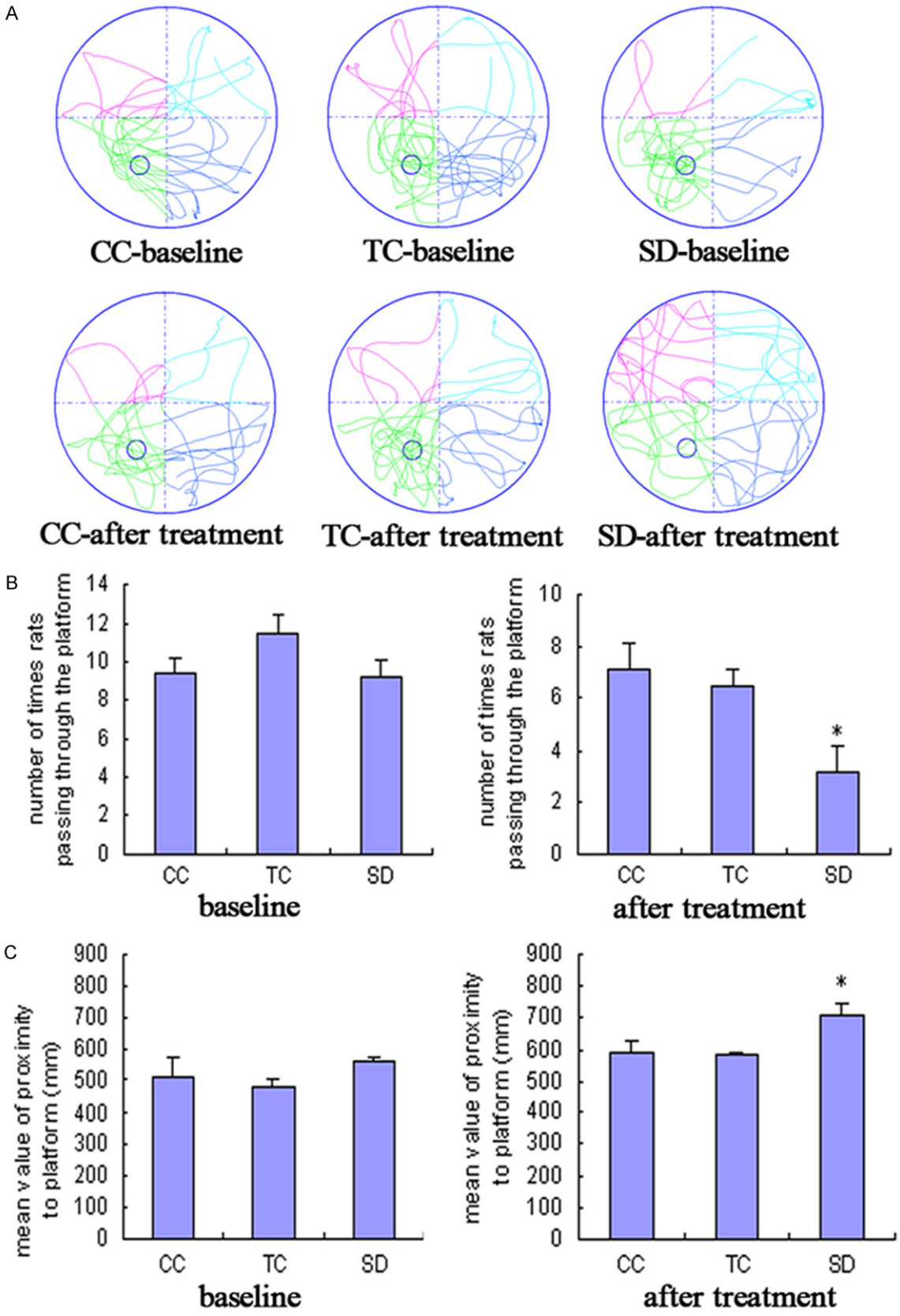

Figure 1. Results of the Morris water maze probe test. A. Typical swim trajectories in the probe test. Upper: baseline; lower: experimental treatments (N=6). B. The number of times of rats passing through the platform during the probe test. This number was decreased significantly in the SD group compared with the CC and TC groups after 72 h of

sleep deprivation (*P<0.05, N=6). C. The mean value of proximity to the platform. This value was greater for rats of the SD group compared to those of the CC and TC groups after 72 h of sleep-deprivation (*P<0.05, N=6).

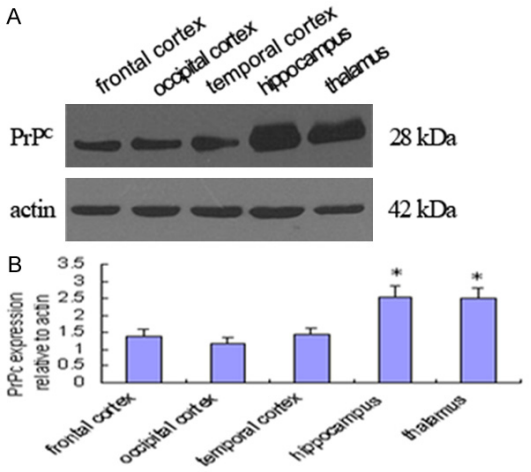

Figure 2. Expression of PrP^C in the normal rat brain. A. PrP^C expression in the frontal cortex, occipital cortex, temporal cortex, hippocampus and thalamus of the normal rat brain was examined by western blotting. b-Actin was used as a loading control (N=6). B. The mean optical density of PrP^C/b-actin. PrP^C expression in the hippocampus and thalamus was significantly higher than its expression in the cerebral cortex (including the frontal cortex, occipital cortex, and temporal cortex) (*P<0.05, N=6).

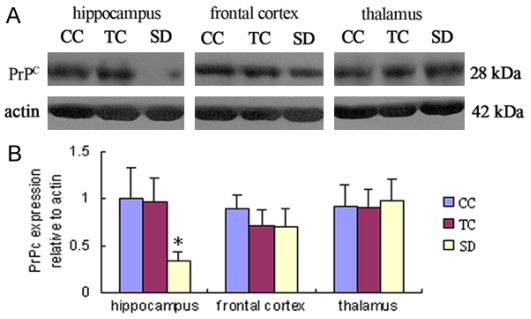

Figure 3. PrP^C expression. A. PrP^C expression was examined by western blotting. PrP^C expression levels in the rat hippocampus, frontal cortex and thalamus after various treatments. b-actin was used as a loading control (N=6). B. The mean optical density of PrP^C/b-actin among the three groups. The data indicate that the hippocampal expression of PrP^C in the SD group sharply decreased after 72 h of sleep-deprivation (*P<0.05, N=6). No changes were observed in other brain regions or groups.

cence staining. The sections were incubated with anti-p-CREB antibody (1:300, Cell Signaling) overnight at 4 degrees centigrade and subsequently incubated with Cy3-conjugated secondary antibody (1:300, Jackson) for 1 h at room temperature. Images were obtained with a Nikon digital camera (DXM1200) attached to a Nikon Eclipse E600 microscope.

Statistical analysis

Significant differences among the treatments were determined using either an analysis of variance (ANOVA) or the Kruskal-Wallis test. A P-value of 0.05 was considered statistically significant.

Results

Sleep-deprivation impairs spatial memory

The swim trajectories of rats in the CC, TC and SD groups during the probe test are shown in **Figure 1A**. After 72 h of sleep-deprivation, rats in the SD group swam randomly throughout the 4 quadrants, whereas the CC and TC groups stayed mainly in the target quadrant. The number of times of rats passing through the platform during the probe test was significantly decreased in the SD group compared with the CC and TC groups (**Figure 1B**, right, P<0.05, n=6). The mean value of proximity to the platform was larger for rats of the SD group compared with the control groups (**Figure 1C**, right, P<0.05, n=6). There were no significant differences in baseline characteristics.

PrP^C expression in various cerebral regions

PrP^C expression in the frontal cortex, occipital cortex, temporal cortex, hippocampus and thalamus of normal rat was detected by western blotting (**Figure 2A**). Results showed that PrP^C expression in the hippocampus and thalamus was significantly higher than that in the cerebral cortex (**Figure 2B**, P<0.05, n=6). We believed that PrP^C must play a critical role in maintaining the normal function of the hippocampus and thalamus. PrP^C expression was examined in three cerebral regions (hippocampus, frontal cortex and thalamus) of CC, TC and SD groups by western blotting analysis (**Figure 3A**). Our results showed that PrP^C expression in the SD group decreased significantly in hippocampus compared with control groups (**Figure 3B**, P<0.05, n=6) after 72 h sleep-deprivation. At same time, no differences were observed in

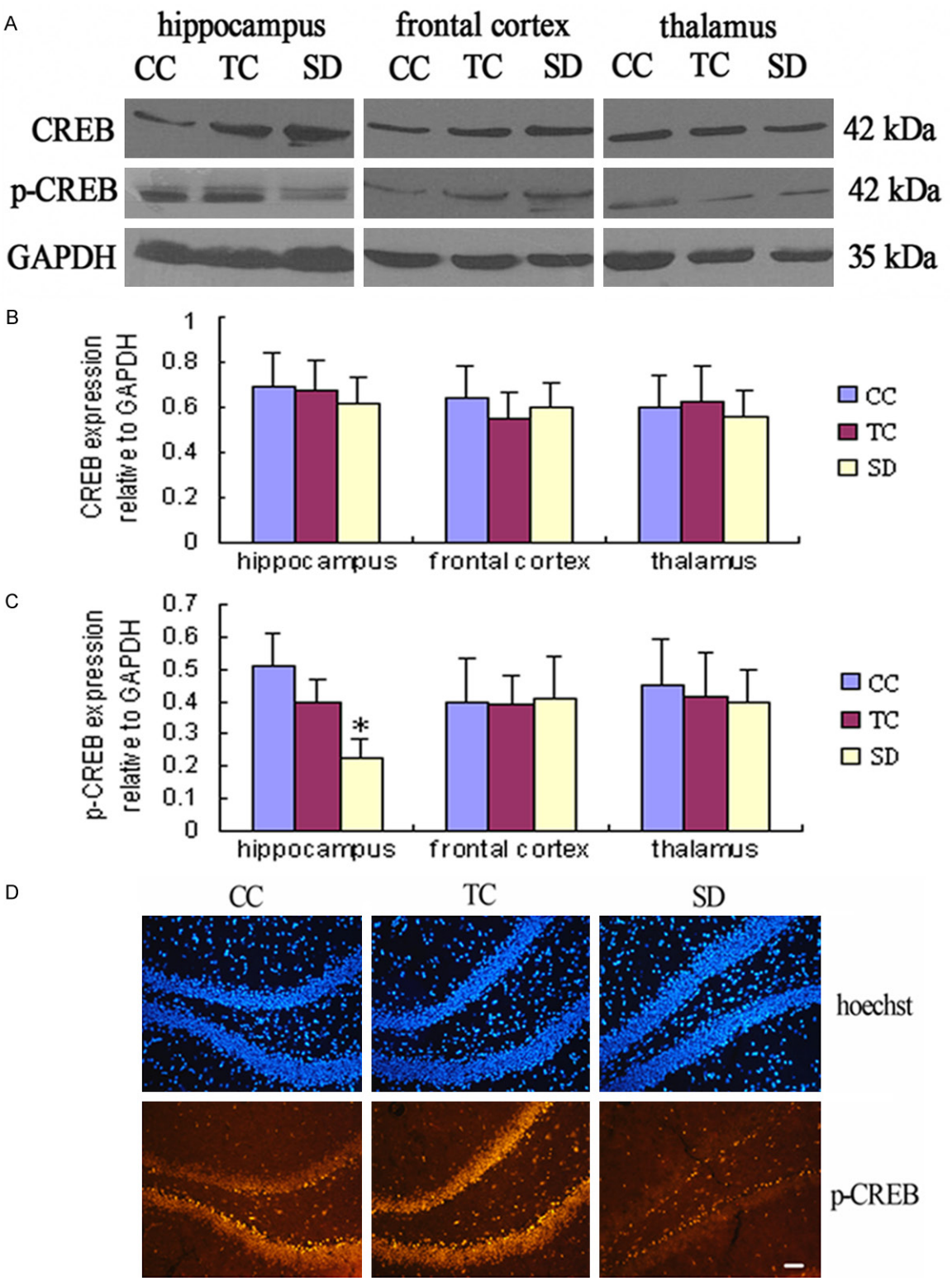

Figure 4. CREB expression and phosphorylation. A. CREB expression and phosphorylation was examined by western blotting. CREB expression and phosphorylation levels in the rat hippocampus, frontal cortex and thalamus after different treatments are shown. GAPDH was used as a loading control (N=6). B. The mean optical density of CREB/GAPDH. No differences were observed among regions or groups (N=6). C. The mean optical density of p-CREB/GAPDH. The phosphorylation of CREB in the hippocampus was obviously suppressed in rats of the SD group after 72 h of sleep-deprivation (*P<0.05, N=6). D. Immunofluorescence experiment. A smaller number of p-CREB positive cells were observed in the dentate gyrus of the hippocampus in the SD group compared with the control groups (N=6).

the frontal cortex or thalamus among the CC, TC and SD groups.

The CREB expression and phosphorylation in various cerebral regions

The expression and phosphorylation of CREB in hippocampus, frontal cortex and thalamus were simultaneously examined using western blotting analysis (**Figure 4A**) in all groups. After 72 h of sleep-deprivation, the CREB phosphorylation in the hippocampus was significantly inhibited (**Figure 4C**, $P<0.05$, $n=6$). A smaller number of p-CREB-positive cells in dentate gyrus of hippocampus were observed in the SD group (**Figure 4D**). But the differences among the groups were no significant after examining hippocampal regions CA1 and CA3 (Data not shown). Additionally, no significant differences were observed in p-CREB-positive cell number in the frontal cortex and thalamus (Data not shown).

Discussion

Although plenty of research has been done, the details of sleep-deprivation induced cognitive impairment still remain unclear. In this study, we confirmed that sleep deprivation reduces PrP^C expression in the hippocampus, which might involve in memory impairment during sleep-deprivation.

Previous experiments using PrP-null mice suggested that PrP^C was not necessary for normal physiological function. However, PrP^C is highly expressed in the nervous system, and its role in neuronal function has been widely concerned. Unfortunately, the role of PrP^C is still not well understood in the past 20 years. Increasing evidence suggests that PrP^C is involved in memory, sleep regulation and some signal transduction pathways [4].

Our data showed that the expression of PrP^C in hippocampus was significantly higher than that in frontal cortex, occipital cortex and temporal cortex in normal rat. That suggests PrP^C might act as a hippocampal-dependent function regulator. But there is evidence that the cerebral cortex displayed a higher level of PrP^C expression than the hippocampus [12]. Thus, more independent studies are necessary in future.

We confirmed that sleep-deprivation can impair the spatial memory of rats indeed, which was similar to conclusions of previous studies [13, 14]. Moreover, we found that PrP^C expression in the rat hippocampus was inhibited. It is well known that hippocampal oxidative stress plays an important role in sleep-deprivation related memory impairment [15, 16]. In view of PrP^C protecting cells from oxidative damage [17, 18], its down-regulation after sleep-deprivation can aggravate oxidative damage and memory impairment in an indirect way. Data show that PrP^C plays a key role in several signaling pathways including the cAMP/PKA, MAPK, PI3-K/Akt and soluble non-receptor tyrosine kinase pathways [4]. Among them, PrP^C can promote neuro-protection or neuritogenesis via PKA or MAPK signaling pathways [19], and modulate memory consolidation via PKA and ERK1/2 pathways [20].

CREB is not only known to involve in hippocampal-dependent learning, memory storage, but also involve in synaptic plasticity [21], oxidative stress [22]. In 1C11 precursor cells and their neuronal progenies, CREB is proved to be target of PrP^C, act roles of cyto-protection and neuronal plasticity [23]. Our results showed that CREB phosphorylation in the hippocampus was inhibited after sleep-deprivation, which was consistent with previous reports [8]. These results suggest that the inhibition of CREB phosphorylation in the hippocampus of sleep-deprived rats may be a result of low PrP^C expression. The simultaneous changes of PrP^C and p-CREB in rat's hippocampus after sleep-deprivation can be observed in our study, which indicate that PrP^C and p-CREB may share some common mechanisms in memory impairment.

In conclusion, we confirmed that sleep-deprivation reduced the expression of PrP^C in the hippocampus, which suggests it contributes to sleep-deprivation induced hippocampal-dependent memory impairment. Moreover, we found CREB might involve in one of the downstream signaling pathways of PrP^C. Thus, PrP^C-CREB may be a potential target for treatment of sleep-deprivation induced memory impairment in future.

Disclosure of conflict of interest

None.

Address correspondence to: Wei Dou, Division of Neurology, 456 Hospital of PLA, Jinan 250031,

China. Tel: +86 053151665470; Fax: +86 531 51666295; E-mail: wei_dou2015@sina.com

References

[1] Rechtschaffen A, Gilliland MA, Bergmann BM and Winter JB. Physiological correlates of prolonged sleep deprivation in rats. Science 1983; 221: 182-184.

[2] Graves LA, Heller EA, Pack AI and Abel T. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem 2003; 10: 168-176.

[3] Guan Z, Peng X and Fang J. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. Brain Res 2004; 1018: 38-47.

[4] Linden R, Martins VR, Prado MA, Cammarota M, Izquierdo I and Brentani RR. Physiology of the prion protein. Physiol Rev 2008; 88: 673-728.

[5] Criado JR, Sanchez-Alavez M, Conti B, Giacchino JL, Wills DN, Henriksen SJ, Race R, Manson JC, Chesebro B and Oldstone MB. Mice devoid of prion protein have cognitive deficits that are rescued by reconstitution of PrP in neurons. Neurobiol Dis 2005; 19: 255-265.

[6] Tobler I, Gaus SE, Deboer T, Achermann P, Fischer M, Rulicke T, Moser M, Oesch B, McBride PA and Manson JC. Altered circadian activity rhythms and sleep in mice devoid of prion protein. Nature 1996; 380: 639-642.

[7] Ulloor J and Datta S. Spatio-temporal activation of cyclic AMP response element-binding protein, activity-regulated cytoskeletal-associated protein and brain-derived nerve growth factor: a mechanism for pontine-wave generator activation-dependent two-way active-avoidance memory processing in the rat. J Neurochem 2005; 95: 418-428.

[8] Vecsey CG, Baillie GS, Jaganath D, Havekes R, Daniels A, Wimmer M, Huang T, Brown KM, Li XY, Descalzi G, Kim SS, Chen T, Shang YZ, Zhuo M, Houslay MD and Abel T. Sleep deprivation impairs cAMP signalling in the hippocampus. Nature 2009; 461: 1122-1125.

[9] Grahnstedt S and Ursin R. Platform sleep deprivation affects deep slow wave sleep in addition to REM sleep. Behav Brain Res 1985; 18: 233-239.

[10] Machado RB, Hipolide DC, Benedito-Silva AA and Tufik S. Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. Brain Res 2004; 1004: 45-51.

[11] Vorhees CV and Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006; 1: 848-858.

[12] Moleres FJ and Velayos JL. Expression of PrP(C) in the rat brain and characterization of a subset of cortical neurons. Brain Res 2005; 1056: 10-21.

[13] Yang RH, Hu SJ, Wang Y, Zhang WB, Luo WJ and Chen JY. Paradoxical sleep deprivation impairs spatial learning and affects membrane excitability and mitochondrial protein in the hippocampus. Brain Res 2008; 1230: 224-232.

[14] Zhao Z, Huang L, Wu H, Li Y, Zhang L, Yin Y and Xiang Z. Neuropeptide S mitigates spatial memory impairment induced by rapid eye movement sleep deprivation in rats. Neuroreport 2010; 21: 623-628.

[15] Silva RH, Abilio VC, Takatsu AL, Kameda SR, Grassl C, Chehin AB, Medrano WA, Calzavara MB, Registro S, Andersen ML, Machado RB, Carvalho RC, Ribeiro Rde A, Tufik S and Frussa-Filho R. Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. Neuropharmacology 2004; 46: 895-903.

[16] Alzoubi KH, Khabour OF, Rashid BA, Damaj IM and Salah HA. The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: the role of oxidative stress. Behav Brain Res 2012; 226: 205-210.

[17] Klamt F, Dal-Pizzol F, Conte da Frota ML Jr, Walz R, Andrades ME, da Silva EG, Brentani RR, Izquierdo I and Fonseca Moreira JC. Imbalance of antioxidant defense in mice lacking cellular prion protein. Free Radic Biol Med 2001; 30: 1137-1144.

[18] White AR, Collins SJ, Maher F, Jobling MF, Stewart LR, Thyer JM, Beyreuther K, Masters CL and Cappai R. Prion protein-deficient neurons reveal lower glutathione reductase activity and increased susceptibility to hydrogen peroxide toxicity. Am J Pathol 1999; 155: 1723-1730.

[19] Lopes MH, Hajj GN, Muras AG, Mancini GL, Castro RM, Ribeiro KC, Brentani RR, Linden R and Martins VR. Interaction of cellular prion and stress-inducible protein 1 promotes neuritogenesis and neuroprotection by distinct signaling pathways. J Neurosci 2005; 25: 11330-11339.

[20] Coitinho AS, Freitas AR, Lopes MH, Hajj GN, Roesler R, Walz R, Rossato JI, Cammarota M, Izquierdo I, Martins VR and Brentani RR. The interaction between prion protein and laminin modulates memory consolidation. Eur J Neurosci 2006; 24: 3255-3264.

[21] Mizuno M, Yamada K, Maekawa N, Saito K, Seishima M and Nabeshima T. CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. Behav Brain Res 2002; 133: 135-141.

[22] Lee B, Cao R, Choi YS, Cho HY, Rhee AD, Hah CK, Hoyt KR and Obrietan K. The CREB/CRE transcriptional pathway: protection against oxidative stress-mediated neuronal cell death. J Neurochem 2009; 108: 1251-1265.

[23] Pradines E, Loubet D, Schneider B, Launay JM, Kellermann O and Mouillet-Richard S. CREB-dependent gene regulation by prion protein: impact on MMP-9 and beta-dystroglycan. Cell Signal 2008; 20: 2050-2058.