Behavioural Brain Research 300 (2016) 70–76

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Short-term sleep deprivation disrupts the molecular composition of ionotropic glutamate receptors in entorhinal cortex and impairs the rat spatial reference memory

Meilan Xie, Chao Li, Chao He, Li Yang, Gang Tan, Jie Yan, Jiali Wang*, Zhian Hu*

Department of Physiology, Collaborative Innovation Center for Brain Science, Third Military Medical University, Chongqing 400038, PR China

HIGHLIGHTS

- Sleep deprivation changed the expression of the entorhinal AMPAR and NMDAR subunits.
- Sleep deprivation impaired rat spatial reference memory.
- Rat emotional activities in open field were not influenced by sleep deprivation.

ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form
28 September 2015
Accepted 2 October 2015
Available online 9 October 2015

Keywords:
Entorhinal cortex
Sleep deprivation
Ionotropic glutamate receptor
Morris water maze

ABSTRACT

Numerous studies reported that sleep deprivation (SD) causes impairment in spatial cognitive performance. However, the molecular mechanisms affected by SD underlying this behavioral phenomenon remain elusive. Here, we focused on the entorhinal cortex (EC), the gateway of the hippocampus, and investigated how SD affected the subunit expression of AMPARs and NMDARs, the main ionotropic glutamategic receptors serving a pivotal role in spatial cognition. In EC, we found 4 h SD remarkably reduced surface expression of GluA1, while there was an increase in the surface expression of GluA2 and GluA3. As for NMDARs, SD with short duration significantly reduced the surface expression levels of GluN1 and GluN2B without effect on the GluN2A. In parallel with the alterations in AMPARs and NMDARs, we found the 4 h SD impaired rat spatial reference memory as assessed by Morris water maze task. Overall, these data indicate that brief SD differently affects the AMPAR and NMDAR subunit expressions in EC and might consequently disrupt the composition and functional properties of these receptors.

1. Introduction

Sleep serves a vital function in metabolic homeostasis, learning-related synapse formation and neuronal reactivation, which is indispensable for the memory formation [1–5]. Numerous studies reported that sleep deprivation (SD) causes impairment in the cognitive performance, especially about the spatial learning and memory. Acute or chronic SD has been reported to affect neuronal function [6] and cause deficits in the acquisition and consolidation of spatial reference memory in Morris water maze [7–11]. In addition, SD impaired the spatial working memory in radial maze [12], T-maze [13] and novel arm recognition tasks [14].

Entorhinal cortex (EC) plays a crucial role in spatial learning and memory [15–17]. Anatomically, it is an interface that mediates the dialogue between hippocampus and neocortex [18,19]. Moreover, EC contains spatially modulated neurons such as grid cells and border cells, and actively participates in spatial information processing [20–22]. Previous studies found that the neurotransmitter receptors in EC are sensitive to SD. Paradoxical SD with 96 h caused a downregulation of the M_2-type cholinergic receptors [23], while the D_1 receptor binding was selectively increased in the EC of SD rats [24].

Among the neurotransmitter receptors, the ionotropic glutamate receptors, especially the 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and *N*-methyl-D-aspartate receptors (NMDARs), mediate the basic excitatory synaptic transmission, and are critical for formation of grid cells in EC and the related spatial cognition [25–28]. AMPARs and NMDARs function as tetramers that are assembled from GluA1-4 and GluN1-3, respectively [25]. In parallel with the SD-induced changes in cognitive function, AMPARs and NMDARs have been reported to be vulnerable to SD in neocortex and

* Corresponding authors.
E-mail addresses: jialiwang@aliyun.com (J. Wang), zhianhu@aliyun.com (Z. Hu).

http://dx.doi.org/10.1016/j.bbr.2015.10.002
0166-4328/

hippocampus. A brief or chronic SD has been reported to alter the AMPARs and NMDARs, especially their subunit expression levels, in these brain regions [13,29–31].

Although the numerous studies have concerned the influence of SD on the hippocampal and cortical AMPARs and NMDARs, whether and how SD affects these subunit expression in the EC remains unexplored. To fully grasp the effects of the SD in spatial memory circuitry, the present study focused on EC and conducted western blotting experiments to systematically detect the effects of SD with short duration (4 h) that frequently occurs in our daily life on the total and surface expressions of AMPAR and NMDAR subunits. Our findings suggest that brief SD disrupted the molecule composition of the AMPARs and NMDARs in EC, which might partially underlie the SD-induced deficits in spatial cognition.

2. Materials and methods

2.1. Animal

Male Sprague-Dawley rats weighting 140 ± 20 g were used in the present study. Rats were housed in a Plexiglas cages (four per cage), with a 12:12 light–dark cycle (lights on at 8:00 a.m.) and with food and water available *ad libitum*. Room temperature was controlled at 22 ± 1°C.

2.2. Sleep deprivation

In the present study, eight animals were randomly divided into control and SD groups. The method for total SD is consistent with that of previous studies [2,13,32]. Briefly, the experimenter introduces novel objects into the Plexiglas cages and gently shakes the cage. If the rats were attempting to sleep, we gently touched the animals with a soft brush. The animals were administrated with SD for 4 h, starting at 8:00 a.m. For the control groups, animal were left in their home cage with no disturbances in the same circumstances.

2.3. Surface biotinylation assays

The methods for surface biotinylation and western blotting assays were similar to our previous report [13]. After the 4 h SD, the brains of animals in the SD-treated and control groups were rapidly removed and sliced at 400 μm thick. The tissue of EC used for the present study is from the same animals that were reported in our previous study [13]. The EC regions were dissected according to the rat brain atlas of Paxinos and Watson. The EC slices in each group (4 animals) were gathered as one sample and labeled with Sulfo-NHS-SS-Biotin in artificial cerebrospinal fluid (ACSF) as described in the previously studies [26]. The slices were first washed with ice-cold ACSF and incubated with Sulfo-NHS-SS-biotin on ice for about 60 min. Then, slices were brief washed with ACSF for three times to remove excessive biotin and lysed in of RIPA buffer (with 1% protease inhibitor cocktail; Sigma) about 60 min. After that, the sample was centrifuged at 10,000 × *g* for 10 min for removal of debris. We harvested the supernatants and quantified it by a BCA protein assay kit (Pierce). The supernatants with equal amounts were incubated with 50% NeutrAvidin agarose (Pierce) overnight. The samples were washed for three times with lysis buffer (with 0.1% protease inhibitor cocktail), and then eluted with 2x SDS sample buffer. Finally, SDS gel electrophoresis and Western blotting analyses were carried out as described below.

2.4. Western blotting assays

The protein samples were separated by 10% polyacrylamide gels and transferred onto polyvinylidene difluoride (PVDF, BioRad) membranes. The following primary antibodies were used: anti-GluA1 (Millipore), anti-GluA2 (Millipore), anti-GluA3 (Millipore), anti-GluN1 (Millipore), anti-GluN2A (Abcam), and anti-GluN2B (Abcam). Then the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Dako) and visualized by the ECL system (Pierce). Densitometry measurements were conducted by using Quantity ONE software (Bio-Rad). Surface and total expression levels of the main AMPAR and NMDAR subunits were normalized to beta-actin loading control.

2.5. Morris water maze test

Animals were administrated with the Morris water maze (MWM) test immediately post 4 h SD. The water maze (50 cm high, 120 cm diameter) was divided into four quadrants. In one of the quadrants, an escape platform (9 cm diameter) was constantly placed about 1 cm below the surface of opaque water (25 ± 1 °C). There were clearly visible cues such as pictures and electronic wires around the water maze as the reference for the orientation. A ceiling-mounted tracking system was used to recode a number of parameters in the water maze, such as the swimming speed, the latency and swimming distance to find the hidden platform. At the training sessions, the animal was put in one of the four quadrants in a quasi-random order across different trials. The animals had to swim until they find the escape platform within 60 s. If they failed to find the escapes platform within 60 s, they were guided to the escape platform by the experimenter. The animals were allowed to stay on the escape platform for about 10 s and break for about 30 s post each trail. The animals were administrated to a total of sixteen trials. The series of 16 trials were averaged for blocks of 4 trails for statistical analysis. After completion of acquisition trials, the platform was removed and a probe trial was conducted, in which the rats were placed at the opposite site to the target quadrant and allowed for freely swimming for 90 s. The performance for probe trial of each group was represented by the proportion of total time spent in each quadrant of the water maze.

2.6. Open field test

After the rat administration of SD, open field test was conducted in a separate group of rats to investigate whether SD altered the rat exploratory and emotional functions. Rat was transferred into the central of a square open field (50 cm × 50 cm × 40 cm) immediately post the 4 h SD and allowed for 3 min freely moving. The parameters including the number of rearing, number of grooming, number of grid crossing, number of visiting the center, and time spent in the central area was recorded by video tracking system. The open field chamber was cleaned with 70% alcohol between different trials.

2.7. Statistical analysis

All data were represented as mean ± S.E.M. The unpaired *t*-test was conducted to analyze the differences of the ionotropic glutamate receptor subunit expression levels across groups. Two-way repeated measures ANOVA was used to test the differences of the escape latency and swimming speed in water maze. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. The surface, instead of total, expression levels of AMPAR subunits were affected by 4 h ***SD***

The EC is regarded as the gateway of the hippocampus and plays an important role in episodic memory, especially spatial memory [16,18]. Here, we first examined effects of SD on EC AMPAR