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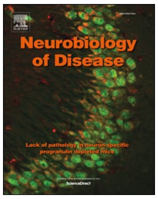

Microglia-mediated synaptic pruning is impaired in sleep-deprived adolescent mice

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ABSTRACT

The detrimental effects of sleep insufficiency have been extensively explored. However, only a few studies have addressed this issue in adolescents. In the present study, we examined and compared the effects of 72 h paradoxical sleep deprivation (SD) on adolescent (5 weeks old) and adult (~12 weeks old) mice. Following 72 h of SD, induced by a modified multiple-platform method, mice were subjected to behavioral, histological and neurochemical examinations. In both adolescent and adult mice, SD adversely affected short-term memory in a novel object recognition test. Compared with normal-sleep controls, sleep-deprived adolescent mice had an increased density of excitatory synapses in the granule cells of the dentate gyrus, but no such pattern was observed in the adult group. The engulfment of postsynaptic components within the microglia after SD was reduced in adolescents but not in adults, suggesting an impaired microglia-mediated synaptic pruning in adolescent SD mice. Possible contributing factors included the decreases in CX3CR1, CD11b and P2Y12, closely associated with the synaptic pruning via microglial phagocytosis. In adult SD mice, microglia-associated inflammatory reactions were noted. In sum, sleep deprivation induces age-dependent microglial reactions in adolescent and adult mice, respectively; yet results in similar defects in short-term recognition memory. Sufficient sleep is indispensable for adolescents and adults.

1. Introduction

Since the evolvement of modern society has become accompanied by the increasing demand for prolonged wakefulness, sleep insufficiency is now recognized as a serious public health issue in adult as well as adolescent population (Broman et al., 1996; Palma et al., 2013). Decreasing sleep duration or sleep quality or both may affect the ability to carry out daily activities and even bring in injurious consequences (Centers for Disease and Prevention, 2011; Garbarino et al., 2004; Mitler et al., 1988). However, while most of the studies focusing on adults, the effects of sleep insufficiency during adolescence period have not been as much addressed. Reports of sleep in adolescents across countries and regions portray a consistent pattern of delayed sleep timing and decreased amounts of sleep due to hormonal circadian shift and early school start times (Bartel et al., 2015; Eaton et al., 2010; Gau and Soong, 1995; Owens et al., 2014). According to a review published by National Sleep Foundation in 2015, 8–10 h of sleep is recommended for adolescents (Hirshkowitz et al., 2015). However, a national survey of US high school students showed that two-thirds of the population reported insufficient sleep on an average school night; such a deficiency is strongly associated with increased health-risk behaviors (McKnight-Eily et al., 2011). The devastating impact of sleep insufficiency in adolescents has also been addressed in experimental and epidemiological studies, which reported that inadequate sleep time led to consequences such as poor academic performance and increased risks for mental health problems including depression, aggression, mood disorder and conduct problems (Moore and Meltzer, 2008; Owens et al., 2014; Shochat et al., 2014; Talbot et al., 2010). Until recently, however,

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Abbreviations: BP, big platform; CX3CR1, CX3C chemokine receptor 1; CX3CL1, chemokine (C-X3-C motif) ligand 1 (Fractalkine); C1qa, complement C1q subcomponent subunit A; C3, complement component 3; DG, dentate gyrus; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HC, home cage; Iba1, ionized calcium binding adaptor molecule 1; IL-1β, Interleukin 1 beta; IL-6, Interleukin 6; NORT, novel object recognition test; PSD95, postsynaptic density protein 95; P2Y12, purinergic receptor P2Y, G-protein coupled, 12; Shank3, SH3 and multiple ankyrin repeat domains 3; SD, paradoxical sleep deprivation; TNF-α, tumor necrosis factor alpha

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few studies had investigated the neurobiological basis of adverse effects of sleep insufficiency in the developing brain (Bellesi et al., 2017; Billeh et al., 2016; Jiang et al., 2017; Maret et al., 2011).

Evidence from animal and human studies has suggested that sleep is essential for proper cognitive performance in domains such as attention, executive function, learning and memory by restoring energy balance and optimizing synaptic plasticity and homeostasis (Abel et al., 2013; Gronli et al., 2013; Krause et al., 2017; McCoy and Strecker, 2011; Tononi and Cirelli, 2014; Wang et al., 2011). The adolescent brain undergoes a set of substantial neurobiological changes, including myelination, synaptic pruning and construction of inhibitory neurotransmission, all contributing to the mature neurocircuitry and, ultimately, to higher cognitive functions (Arain et al., 2013; Casey et al., 2008; Paus et al., 2008). Recent studies have suggested that the high remodeling activity in the adolescent brain may increase its susceptibility to acute or chronic sleep insufficiency. For example, after 4 days of sleep restriction, the cortical neurons of adolescent mice exhibit long-term ultrastructural changes in mitochondria and components of the endocytotic pathway even after recovery sleep (de Vivo et al., 2016). Moreover, after 5 days of sleep restriction in adolescent mice, a subtle yet long-lasting change in secondary motor cortex connectivity was noticed (Billeh et al., 2016). These studies support the notion that sleep disruption in the early years of life may impair brain maturation and render long-term consequences. However, detailed studies are needed to reveal how sleep insufficiency affects the adolescent nervous system structurally and functionally.

Due to the intimate interaction between glia and neurons during sleep (Bellesi et al., 2015; Fellin et al., 2012; Halassa et al., 2009) and the altered immune responses brought by sleep loss (Irwin et al., 2016; Mullington et al., 2010), microglia have attracted our attention. Previous studies have shown that sleep disturbance results in elevated levels of proinflammatory cytokines (Irwin et al., 2006; Zhu et al., 2012), morphological changes in microglia and suppression of nitric oxide-synthesizing enzymes (Hsu et al., 2003). Inhibition of microglial activity by minocycline results in prevention of both sleep pressure build-up (Wisor et al., 2011) and spatial memory defects (Wadhwa et al., 2017) after sleep deprivation. Moreover, chronic prolonged wakefulness increases astrocytic phagocytosis and microglial priming in the cortex of young mice (Bellesi et al., 2017). These results demonstrate that microglia passively respond to sleep deprivation by exiting their normal quiescent state and that they may pose a threat to the sleep-deprived brain. Apart from their well-characterized role in innate immunity, microglia also participate in modulating synaptic refinement by surveying and selectively phagocytosing pre- and postsynaptic elements in a neuronal activity-dependent manner in the healthy developing brain (Chung et al., 2015; Kettenmann et al., 2013; Neniskyte and Gross, 2017; Sierra et al., 2013). Accumulating evidence of microglial involvement in the adolescence synaptic pruning, a marked feature of neurodevelopment, has been reported in various brain regions and are essential for the function of neural circuits (Kopec et al., 2018; Mallya et al., 2019; Parkhurst et al., 2013). Furthermore, defects of microglia surveillance and phagocytic function during development have been demonstrated to result in neural circuit dysfunction and even psychopathological illnesses such as autism (Kim et al., 2017; Schafer et al., 2012; Zhan et al., 2014). Based on those evidence, we hypothesized that microglia functions in adolescent and adult brains may be overshadowed by sleep insufficiency.

Here, we sought to address the role of microglia in the deleterious impact of sleep deprivation on the brain during developmental organization as well as achieved maturation. In this study, we induced 72 h SD through a modified multiple-platform method and conducted histological, neurochemical and behavioral examinations following SD in adolescent and adult mice. We present evidence that SD-induced impairment of synaptic pruning is attributable to the decline of microglial phagocytic capacity in the adolescent brains. On the other hand, our results indicated that microglial response predisposed to neuroinflammation by SD in the adult brains, which in resonance with previous research on SD-invoked inflammatory effects (Hsu et al., 2003; Wadhwa et al., 2017; Wisor et al., 2011; Zhu et al., 2012). Taken together, the current research demonstrated that SD perturbed microglial homeostasis differentially in adolescent and adult mice.

2. Methods

2.1. Animals

Adolescent (5 weeks old) and adult (~12 weeks old) male mice of C57BL/6 J background were obtained from the National Laboratory Animal Center, Taiwan, and randomly assigned to the SD, big platform (BP) control and home cage (HC) control groups. The mice were housed in standard plastic home cages under a 12 h/12 h light/dark cycle and received ad libitum standard rodent chow and water. All animal experiments were performed in accordance with the guidelines set by the Institutional Animal Care and Use Committee of the College of Medicine, National Taiwan University.

2.2. 72 h SD paradigm

SD for 72 h was induced using a modified multiple-platform method in a water tank. In brief, mice in the SD group were placed on small platforms (3 cm in diameter) surrounded by water and allowed to move freely from one platform to another. In the BP control group, mice were placed on platforms of 10 cm in diameter instead. SD started at the beginning of a dark phase. Food pellets were freely available for each group.

2.3. Behavioral tests

The behavioral examinations were conducted during the dark phase 2 h after the end of 72 h SD and completed within 2 h. Before testing, the mice were placed in the experimental environments for habituation (> 30 min). After each test, the apparatus was carefully cleaned with 10% alcohol.

2.3.1. Open field test

A square white Plexiglas box with floor dimensions of 45 cm × 45 cm and a wall height of 50 cm was used as an open field apparatus. After habituation, each mouse was placed in the apparatus and allowed to freely explore the arena for 30 min. The activities of mice were continuously videotaped and subsequent analyzed using TopScan software (CleverSys, Reston, VA, USA). The arena was equally divided into 25 squares. The 16 squares adjacent to the walls were designated as the peripheral area, while the central 9 squares were designated as the central area.

2.3.2. Novel object recognition test (NORT)

The NORT was performed immediately after the open field test. Two identical objects were presented, and the mouse was allowed to freely explore for 10 min. After a 10 min intertrial interval, the mouse was retested in the same apparatus, where one of the objects had been replaced by a novel one. Exploration behavior was defined as turning the nose toward the object at a distance ≦2 cm or touching it with the nose, whereas passing by, climbing or sitting on the object was not included. The discrimination index was defined as the percentage of time spent exploring the novel object / total amount of time spent exploring both objects.

2.3.3. Elevated plus maze test

The maze was elevated 50 cm above the floor and consisted of two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm, enclosed by 20 cm-high walls on both sides) extending from a central platform (5 cm × 5 cm), with identical arms positioned opposite to each other. During the test, each mouse was placed on the central platform and allowed to move freely for 10 min. The activities of mice were continuously videotaped with a camera placed above the maze, and the videos were analyzed with the TopScan software (CleverSys).

2.4. Histological examinations

Mice were overdosed by 150 mg/kg sodium pentobarbital and transcardially perfused with 0.1 M PBS followed by 4% paraformaldehyde. Whole brains were harvested and postfixed overnight in the same fixative. For morphometric analyses of neuron and microglia, brains were collected from mice that had completed the behavioral tests. For c-fos analysis, mice of another cohort were sacrificed and perfused 2 h after the NORT test. For PSD95 puncta and microglia engulfment assays, the other cohort of mice were sacrificed and perfused right after the 72 h SD paradigm. Brain slice from bergma −1.46 mm to −2.46 mm were examined.

2.4.1. Golgi-Cox impregnation and morphometric analyses

Brain samples were immersed in the impregnation solution of FD Rapid Golgi Stain kit (FD NeuroTechnologies, Columbia, MD, USA) and processed as previously described (Juan et al., 2014). In brief, impregnated samples were cut coronally at a thickness of 150 μm using a vibratome (VT 1000S, Leica, Wetzlar, Germany). Sections were then incubated with a mixture of developer and fixer solutions, washed and mounted. For the reconstruction of the granule cells in the dentate gyrus (DG), z-stacks were taken with the StereoInvestigator system (MicroBrightField Bioscience, Williston, VT, USA) at 20× magnification using 1.5 μm z-steps. Morphological reconstruction and analyses were performed using the Neurolucida software (MicroBrightField Bioscience). For dendritic spine examination, images of medial and distal part (> 50 μm away from soma) of dendritic segments were acquired at 100× magnification with a light microscope (DM750, Leica) and analyzed with ImageJ software (NIH, Bethesda, MD, USA).

2.4.2. Immunohistochemistry

Sections were cut at a thickness of 30 μm with a vibratome (Leica), reacted with 1% H_2O_2 to block the endogenous peroxidase activity and transferred to a PBS-based blocking solution containing 4% normal goat serum, 4% bovine serum albumin and 0.4% Triton X-100. After blocking, sections were incubated with rabbit anti-Iba1 antibodies (1:1000, GeneTex, Irvine, CA, USA.) overnight at 4 °C. After washes, sections were incubated with biotinylated goat anti-rabbit IgG (1:1000, Jackson Immuno Research Laboratories, West Grove, PA, USA) and avidin-biotin peroxidase complex (ABC kit, Vector Labs, Burlingame, CA, USA). Finally, sections were reacted with 3,3′-diaminobenzidine (with 0.01% H_2O_2 in PBS) to reveal the target protein.

To evaluate the density of microglia, the areas of the DG molecular layer were measured using the ImageJ software (NIH) and Iba1-positive microglial cells within were counted.

2.4.3. Reconstruction of microglia and morphometric analyses

Z-stacks of microglia in the DG molecular layer were taken with the StereoInvestigator system (MicroBrightField Bioscience) at 40× magnification using 1 μm z-steps. Microglia with definite cell bodies and processes were reconstructed and analyzed with Neurolucida software (MicroBrightField Bioscience).

2.4.4. Immunofluorescence

Coronal sections of 30 μm thick were transferred to the blocking solution as described above and then reacted with diluted primary antibodies, including rat anti-CD68 (1:500, Bio-Rad, Hercules, CA, USA), rabbit anti-c-fos (1:1000, Cell Signaling, Danvers, MA, USA), mouse anti-GFAP (1:1000, Sigma-Aldrich, St. Louis, MO, USA), rabbit anti-Iba1 (1:1000, GeneTex), and mouse anti-PSD95 (1:500, Thermo Fisher Scientific, Waltham, MA, USA), overnight at 4 °C. After washes in PBS, sections were incubated with fluorophore-conjugated secondary antibodies including: Alexa-488 goat anti-mouse IgG, Alexa-594 goat anti-rabbit IgG (1:500, Jackson Immuno Research Laboratories), Alexa-488 goat anti-rat IgG, and Alexa-647 goat anti-rat IgG (1:500, BioLegend, San Diego, CA, USA) for 1 h at room temperature. Finally, sections were mounted in Fluoromount-G (plus DAPI, Southern Biotech, Birmingham, AL, USA).

To evaluate the density of c-fos, the areas of the DG granular layer were measured using the ImageJ software (NIH) and c-fos-positive cells within were counted.

2.4.5. Quantification of postsynaptic puncta

For analysis of PSD95 puncta, 0.9 μm-thick sections containing the DG were acquired with an LSM880 confocal microscope (Carl Zeiss, Oberkochen, Germany) at 63× magnification. The density of PSD95 puncta within the inner (< 40 μm from soma), middle (40–80 μm from soma) and outer (> 80 μm from soma) parts of the molecular layer were quantified via Synapse Counter (plug-in for ImageJ, http://github.com/SynPuCo/SynapseCounter/) (Lin et al., 2016).

2.4.6. Microglial engulfment and CD68 expression

For analysis of PSD95 within microglia or PSD95 within microglial CD68, z-stacks of individual microglia in the molecular layer were acquired with LSM880 at 63× magnification with a 1.5× digital zoom using 0.6 μm z-steps. For analysis of CD68 expression per microglia, z-stacks were acquired at 63× magnification with a 0.7× digital zoom using 0.6 μm z-steps. Images were subsequently processed and analyzed using established methods (Schafer et al., 2014). In brief, the background signal of all fluorescence channels was subtracted using ImageJ (NIH) software. 3D surfaces of microglia and PSD95 puncta/CD68 were created by Imaris software (Bitplane, Zurich, Switzerland) to determine the volume. Analysis thresholds were held constant for all z-stacks. The engulfment volume was expressed as the percentage of PSD95 puncta /CD68 volume within the volume of microglia.

2.5. Biochemical analyses

Mice that had not been used for behavioral tests were decapitated, and hippocampal tissue samples were collected, rinsed in ice-cold PBS, and stored at −80 °C until further analyses.

2.5.1. RT-PCR

Total RNA was isolated with TRIzol™ Reagent (Thermo Fisher Scientific), and 500 pg of total RNA was taken for cDNA synthesis, which was conducted using the Magic RT Mastermix cDNA Synthesis Kit (Bio-Genesis Technologies, Taipei, Taiwan). Quantitative RT-PCR for genes of interest was performed with a SYBR Green Kit (Bio-Genesis Technologies) using 1 μl of cDNA per reaction and was run on a PCR machine (QuantStudio3, Thermo Fisher Scientific) according to the manufacturer's protocols. Expression level of gene of interest was calculated by $\mathrm{ddC_t}$ method normalized to Gapdh. Primers pairs for gene of interest were used as follows. C1q: Forward 5′- CAAGGACTGAAGGGCGTGAA-3′, Reverse 5’-CAAGCGTCATTGGGTTCTGC-3′; C3: Forward 5’-TCAGATAAGGAGGGGCACAA-3′, Reverse 5’-ATGAAGAGGTACCCACTCTGGA-3′; CD11b: Forward 5’-TGGCCTATACAAGCTTGGCTTT-3′, Reverse 5’-AAAGGCCGTTACTGA GGTGG-3′; CSF1R: Forward 5’-GCAGTACCACCATCCACTTGTA-3′, Reverse 5’-GTGAGACACT GTCCTTCAGTGC-3′; CX3CL1: Forward 5’-CCGCGTTCTTCCATTTGTGT-3′, Reverse 5’-GCA CATGATTTCGCATTTCG-3′; CX3CR1: Forward 5’-CAGCATCGACCGGTACCTT-3′, Reverse 5’-GCTGCACTGTCCGGTTGTT-3′; GAPDH: Forward 5’-ACGGGAAACCCATCACCAT-3′, Reverse 5’-CCAGCATCACCCCATTTG A-3′; IL-1 Reverse 5’-TTCTCTGCAGACTCAAACTC CAC-3′; IL-6: Forward 5’-GCTACCAAACTGGATATAATCAGGA-3′, Reverse 5’-CCAGGTA GCTATGGTACTCCAGAA-3′; MEGF-10: Forward 5’-ACTGGAGCCTTCTGTGAGGA-3′, Reverse 5’-ACACTGGCATTCTTGGGAAC-3′; Mertk: Forward 5’-CACAATGACAAAGGACTG ACG-3′, Reverse 5’-AGT

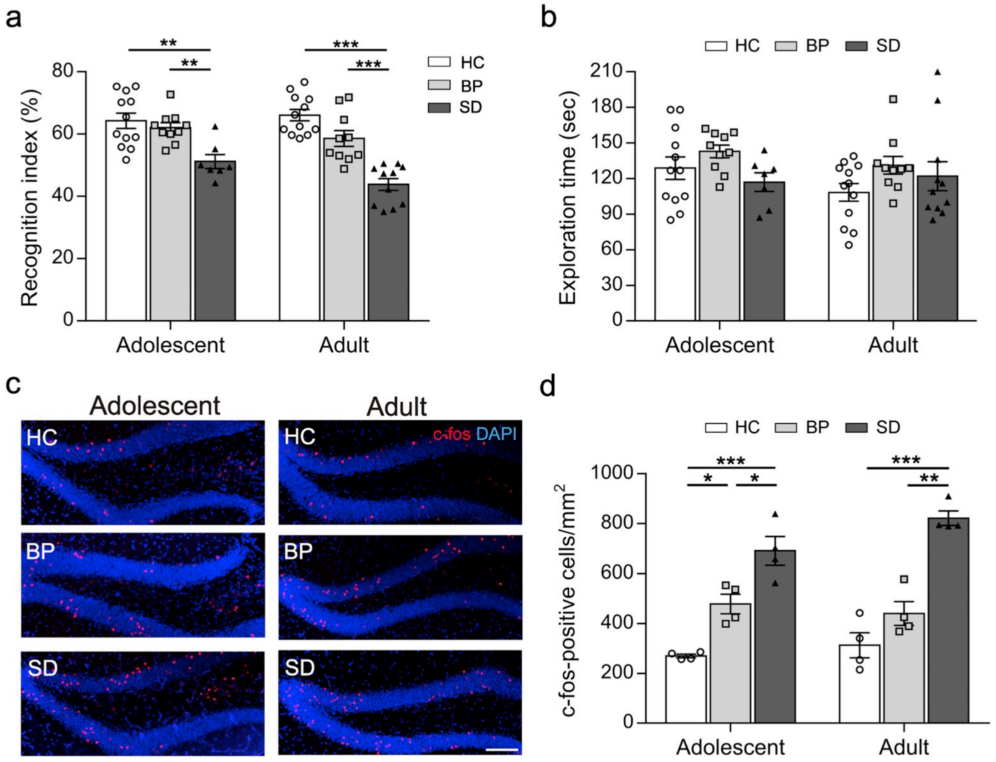

Fig. 1. Performance of short-term memory and hippocampal activity. The short-term memory of mice in the home cage (HC) control, big platform (BP) and sleep deprivation (SD) groups in adolescence and adulthood was evaluated by the novel object recognition test (NORT). Performance was impaired in SD groups of both ages (a), while the total exploration time for both objects was comparable between groups at both ages (b). $n = 7$–12 mice per group. c-fos-positive cells in the DG granular cell layer were revealed by immunohistochemistry 2 h after the NORT (c) Scale bar: 100 μm. The density of c-fos-positive cells increased in the BP and SD groups in adolescent mice and in the SD group in adult mice (d). $n = 4$ mice per group. Asterisks indicate significant differences between groups (* $p < .05$; ** $p < .01$).

AGCCATCAAAACCAGGG-3′; P2Y12: Forward 5'-GTGTTGACACCA GGCACATC-3′, Reverse 5'-TCCCGGAGACACTCATATCC-3′; TNF-α: Forward 5'-AGGCTGC CCCGACTACGT-3', Reverse 5'-GACTTTCTCCTG GTATGAGATAGCAAA-3'.

2.5.2. Western blot

Hippocampal samples were homogenized in RIPA buffer (GenStar Biosolutions, Beijing, China) with a protease inhibitor cocktail (Thermo Fisher Scientific) and centrifuged at 12,000 rpm for 10 min at 4 °C. Supernatants were collected and boiled with Laemmli sample buffer for 5 min. The protein concentration in the supernatant was determined using a bicinchoninic acid assay (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific). Equal amounts of protein samples were loaded and separated by 8% SDS-PAGE and transferred to PVDF membranes (Immobilon®-P, Millipore, Burlington, MA, USA). The membranes were then incubated with diluted primary antibodies including, rabbit anti-PSD95 (1:2000, Abcam, Cambridge, UK), mouse anti-SHANK3 (1:1000, Millipore) or rabbit anti-GAPDH (1:20000, GeneTex) overnight, followed by peroxidase-conjugated secondary antibodies (1:2000, goat anti-rabbit IgG, goat anti-mouse IgG, Vector Laboratories) for 1 h at room temperature. The immunoreactive bands were visualized by chemiluminescent HRP substrate (Immobilon™ Western, Millipore) with a UVP AutoChemi™ System (UVP Inc., Upland, CA, USA). The intensity of the immunoreactive bands was determined with ImageJ (NIH). The expression levels of the proteins of interest were examined relative to GAPDH in a semiquantitative manner.

2.6. Statistical analysis

All data were analyzed by one-way ANOVA followed by post hoc Scheffe's tests using SPSS and expressed as the mean ± SEM. $p < .05$ was considered to indicate a significant difference.

3. Results

The detrimental effects of sleep loss have been demonstrated by a large body of molecular, electrophysiological, anatomical and behavioral evidence (Havekes et al., 2012; Kreutzmann et al., 2015; Prince and Abel, 2013). Most of the findings were obtained from adult subjects; only a few authors have addressed this issue in adolescents (Bellesi et al., 2017; Billeh et al., 2016; Jiang et al., 2017; Maret et al., 2011). In the present study, we examined and compared the effects of 72 h SD on adolescent (5 weeks old) and adult (~12 weeks old) mice.

4. Impact of 72 h SD on behaviors

4.1. Locomotor activity and anxiety level were not affected by SD

We first elucidated the effects of insufficient sleep on behavioral performance. Mice of both age classes in the HC, BP and SD groups were tested in an open field apparatus for 30 min. Locomotor activities, represented by total distance traveled, were comparable among groups in adolescent and adult mice (Fig. S1a). The distance traveled and the time spent in the central area, indexes of anxiety level (Carola et al., 2002), were also comparable among groups at both ages (Fig. S1 a and b). This notion was validated by the elevated plus maze test, in which the level of anxiety can be estimated by the time spent in the open arms (Carola et al., 2002; Prut and Belzung, 2003). These measures were not different between groups at either age (Fig. S1c). Together, these results indicated that the consequences of SD, such as stress and fatigue, might not influence the locomotor activity or anxiety level of mice.

4.2. 72 h SD impaired short-term memory yet enhanced c-fos expression in mice of both ages

One of the major consequences of sleep loss is a deficit in learning and memory (Abel et al., 2013; Krause et al., 2017; McCoy and Strecker, 2011). In this study, the novel object recognition test (NORT) with 10 min intertrial interval, in which proper hippocampal function is required (Cohen et al., 2013), was used to assess the performance of short-term memory. The present results showed that SD profoundly affected short-term memory in SD groups of both ages, as manifested by a significantly reduced recognition index (Fig. 1a), while the total exploration time was comparable between groups at both ages (Fig. 1b).

We further evaluated the expression of c-fos protein, an informative neuronal immediate-early gene regulated by neural activity and associated with learning and memory (Albasser et al., 2010; Guzowski et al., 2001). Under the basal condition, the levels of c-fos expression in the DG granule cells were comparable among the HC, BP and SD groups in both adolescent and adult mice (Fig. S2). Interestingly, after NORT, a significant increase in c-fos expression was observed in both the adult and the adolescent SD groups relative to the HC groups (Fig. 1 c and d), indicating an altered neural activity pattern in SD group of both ages. In BP groups, increased c-fos expressions were also observed, while the significance was not reached in adult group (Fig. 1d).

5. Impact of 72 h SD on DG granule cells

5.1. Subtle decrease in dendritic complexity after SD in adolescent mice

Since 72 h SD affected short-term recognition memory and the activation pattern of DG granule cells, we next examined the structural features of these cells. The morphometric parameters of dendritic arbors were assessed from the 3D-reconstructed Golgi-stained DG granule cells (Fig. 2 a -c). A decrease in the number of bifurcation nodes and number of terminal endings was observed in the SD group of adolescent mice but not in adult mice (Table S1). A subtle change in the dendritic complexity was found in the SD group of adolescent mice, while no difference was noticed between groups of adults (Fig. 2 d-g; Fig. S3).

5.2. An increase in excitatory synapses after SD in adolescent mice

The structure and function of synapses are modulated by wake and sleep in both young and adult subjects (de Vivo et al., 2017; Diering et al., 2017; Havekes et al., 2016; Li et al., 2017; Maret et al., 2011; Vyazovskiy et al., 2008). Accordingly, we examined the density and size of dendritic spines in Golgi-stained DG granule cells (Fig. 2h). In adolescent mice, 72 h SD resulted in an increase in spine density and a decrease in the width of spine heads, whereas no change was observed with SD in adult mice (Fig. 2 i and j).

Since the dendritic spines receive mostly excitatory inputs (Amaral et al., 2007), we examined the density of postsynaptic density protein

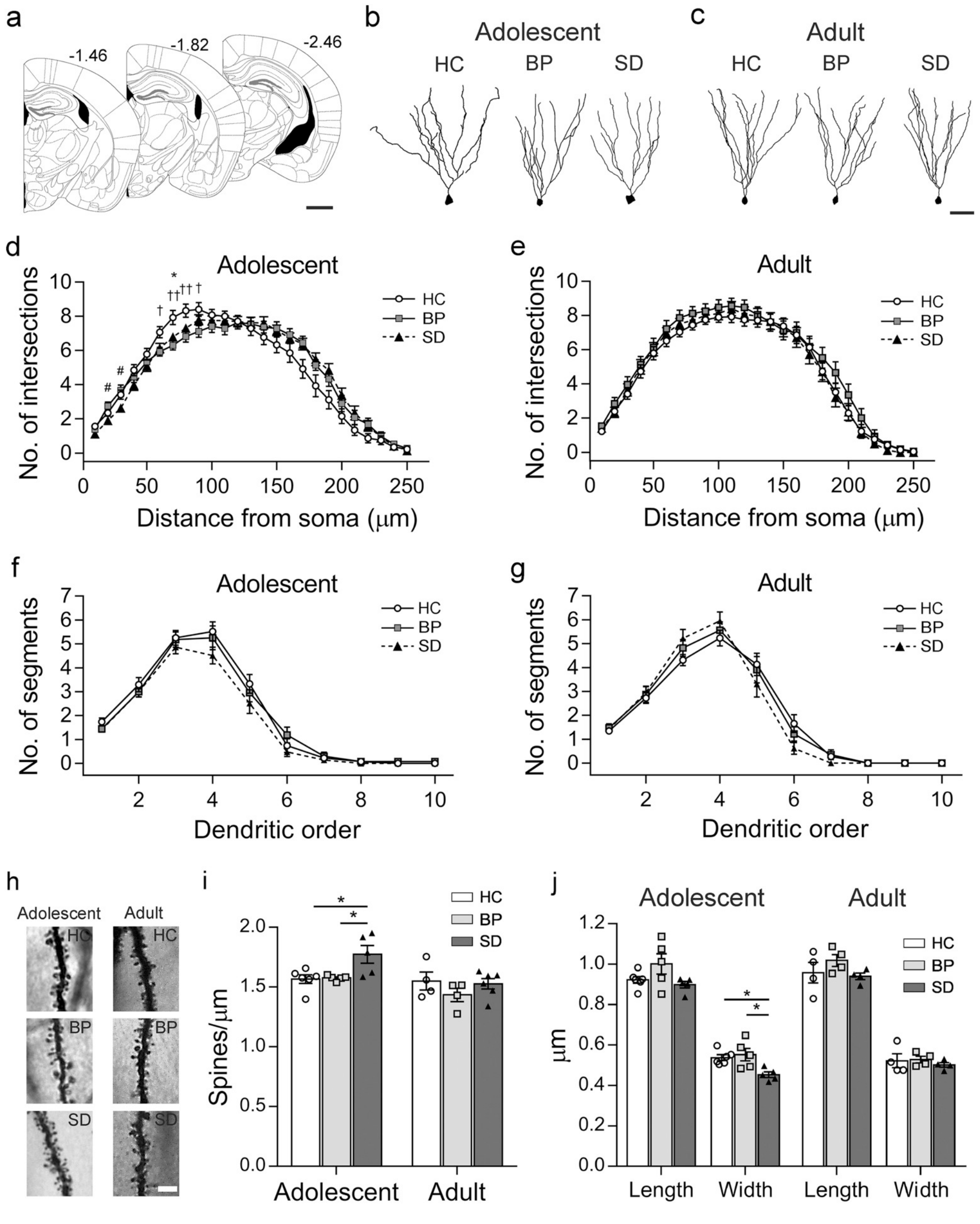

Fig. 2. Morphometric analyses of DG granule cells. Sections containing the dentate gyrus (DG, marked in gray) were collected (a). Golgi-Cox-impregnated DG granule cells in adolescent (b) and adult (c) mice were reconstructed. The complexity of dendritic branches was evaluated by the concentric method of Sholl (d and e) and by the numbers of segments (f and g). *, # and †, respectively, indicate significant differences between HC and SD, between BP and SD, or between HC and BP (*, #, † $p < .05$; †† $p < .01$). The dendritic segments (111–265 of total dendritic segments from 4 to 6 mice per group) were collected and analyzed (h). The density of dendritic spines was examined (i). Furthermore, the length and width of the spine heads of individual spines (417–824 spines from 4 to 6 mice per group) were measured (j). Asterisks indicate significant differences between groups (* $p < .05$). Scale bars: 1 mm in a, 25 μm in b and 5 μm in c. HC, home cage; BP, big platform and SD, sleep deprivation.

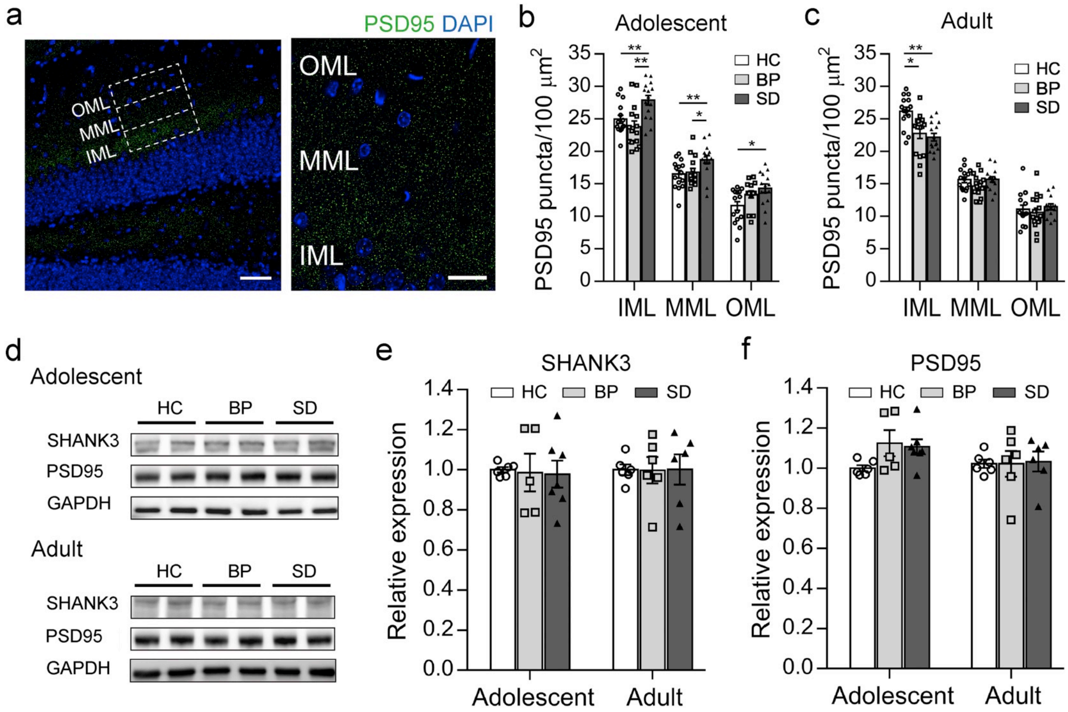

Fig. 3. Expression of postsynaptic proteins in the DG molecular layer and hippocampal homogenates. The density of PSD95 puncta in the DG molecular layer was quantified (a-c). Scale bar: 50 µm in the left, 20 µm in the right. $n = 15$ images from 3 mice. Protein levels of SHANK3 and PSD95 were evaluated by western blot assays (e-g). HC, home cage; BP, big platform and SD, sleep deprivation. $n = 5–6$ mice per group. Asterisks indicate significant differences between groups (* $p < .05$; ** $p < .01$).

95 (PSD95) as an index of excitatory synapses in the inner, middle and outer regions of the molecular layer of the DG (Fig. 3a). In adolescent mice, increased numbers of PSD95 puncta were noticed in all three regions of the molecular layer following SD (Fig. 3b). In adult mice, decrease in PSD95 puncta was observed in the inner region of the molecular layer in the BP and SD groups compared with controls, but no change was found in other regions (Fig. 3c).

PSD95, Shank3 (SH3 and multiple ankyrin repeat domains 3), and other scaffolding proteins form the postsynaptic density by binding the glutamate receptors on the postsynaptic membrane. Thus, the size of the postsynaptic density and the content of PSD95 may determine the synaptic strength (Sheng and Kim, 2011). With semiquantitative protein analysis, the protein levels of PSD95 and Shank3 in the hippocampal tissue were found to be comparable among all three groups at both ages (Fig. 3 d-f). Collectively, these results indicated that instead of a net upregulation of the postsynaptic element, 72 h SD may result in excess of small and weak synapses in adolescent mice.

6. Impact of 72 h SD on microglia

6.1. Microglia density, morphology and proinflammatory cytokines

Activation of parenchymal microglia, accompanied by elevated secretion of proinflammatory mediators, is closely linked to altered synaptic structure and plasticity in neurodevelopmental and neurodegenerative diseases (Mottahedin et al., 2017). In addition, the proinflammatory effect of sleep loss is validated by a large body of human and animal studies (McEwen, 2006; Mullington et al., 2010). In light of this information, we assessed the density and morphology of Iba1-positive microglial cells in the DG molecular layer (Fig. S4a). There was no difference in the density of microglia among the three groups at either age (Fig. S4 b and c). We also quantified the mRNA level of colony-stimulating factor 1 receptor (CSF1R), which is crucial for the proliferation, differentiation and survival of microglia in the brain (Elmore et al., 2014; Erblich et al., 2011). The levels of CSF1R mRNA were comparable between the three treatment groups in both adolescent and adult mice (Fig. S5), indicating the number of microglia is not altered by experimental manipulations.

We further reconstructed Iba1-labeled microglia for morphometric analysis (Fig. 4 a-c). Unexpectedly, 72 h SD produced significant decreases in the number of branch points, number of terminal endings and total length of microglia in adult but not adolescent mice (Table 1). The complexity of microglial processes was reduced after SD in adults, as manifested by decreased numbers of intersections, nodes, ends (Sholl analysis) and segments, while only subtle changes were noticed in the SD group of adolescent mice (Fig. 4 d-g; Fig. S6).

As the retraction of microglial processes has been reported in neuroinflammation models (Kondo et al., 2011), we further quantified the mRNA levels of proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), in hippocampus homogenate. We found no changes after SD in adolescent mice, in contrast to significant increases in TNF-α and IL-6 mRNA expression after SD in adult mice (Fig. 5). These results suggested that 72 h SD induced a proinflammatory response in adult but not adolescent mice. These results indicated altered neuroinflammation status in adult SD mice; however, these changes might not be responsible for impaired synaptic sculpting in sleep-deprived adolescent mice.

6.2. Decreased engulfment of PSD95 and phagocytic capacity in microglia after SD in adolescents

In addition to their role as passive immune sentinels, microglia also contribute to the pruning of synapses by phagocytosis (Kettenmann et al., 2013; Miyamoto et al., 2013; Neniskyte and Gross, 2017; Siskova and Tremblay, 2013). To test the hypothesis that SD disrupts microglia-mediated synaptic pruning in adolescent mice, we acquired z-stacks of immunofluorescence-labeled PSD95 (postsynaptic element) and Iba1 (microglia) in the molecular layer of the DG. Subsequently, 3D surface volume rendering was performed to quantify the internalized PSD95-

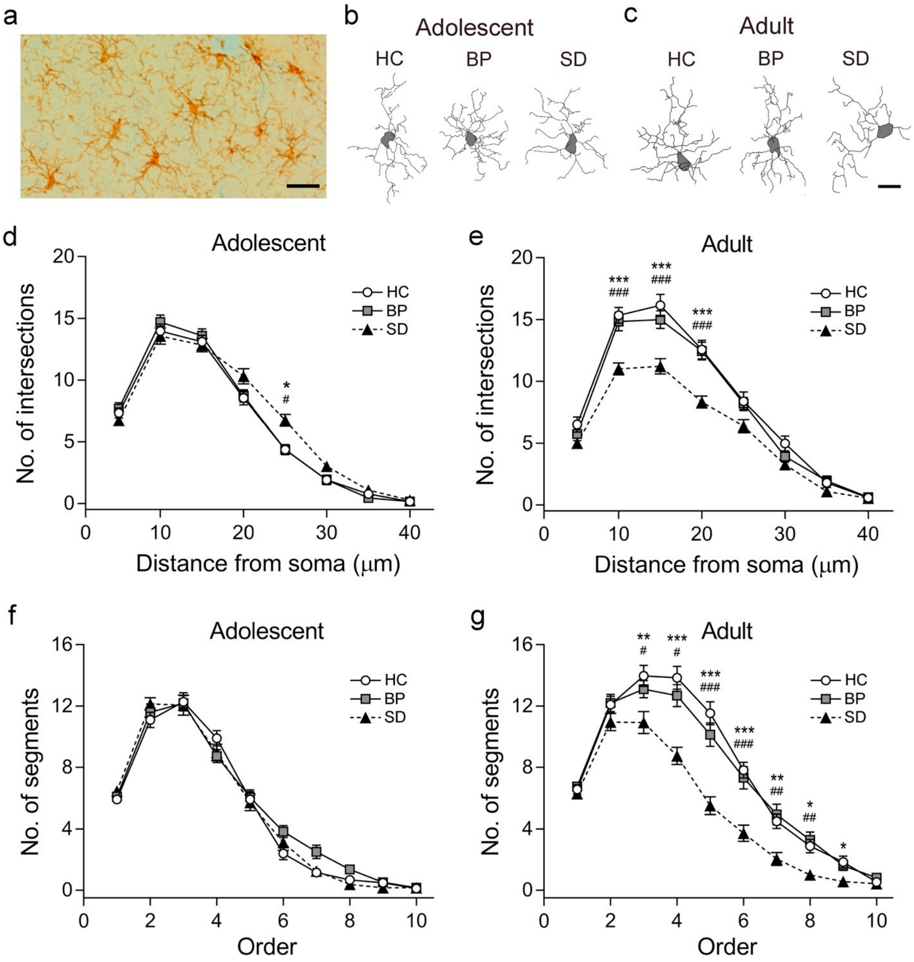

Fig. 4. Morphometric analyses of microglia in the molecular layer of the DG. Microglia were labeled with Iba1 (a) and reconstructed (b, c). Scale bar: 100 μm in a, and 10 μm in c. The complexity of microglial processes (n = 28–39 cells from 3 to 4 mice per group) were analyzed by the concentric-shell method of Sholl (d and e) and by numbers of segments (f and g). HC, home cage; BP, big platform and SD, sleep deprivation. * and #, respectively, indicates significant differences between HC and SD or between BP and SD (*, # $p < .05$; **, ## $p < .01$; ***, ### $p < .001$).

positive puncta within each of the microglia (Fig. 6a). We observed a decreased volume of PSD95 puncta within microglia after SD in adolescents but not in adults (Fig. 6 b and c). Negative control of immunofluorescence experiment without applying PSD95 primary antibody was also conducted (Fig. S7). The false positive signals caused by secondary antibody binding to non-specific IgG could be excluded.

As a lysosomal-associated membrane glycoprotein, CD68 is localized primarily on the lysosomal membrane and is commonly used as an index for the phagocytic capacity of microglia (Schafer et al., 2012; Sierra et al., 2013). Next, we validated the expression of CD68 within individual microglia (Fig. 6d). In adolescent mice, reduced CD68 expression within microglia was found in the SD group, whereas no significant change was found in adult mice (Fig. 6 e and f). Decreased microglia phagocytosis of postsynaptic materials after SD in adolescent was further proven by a significant reduction of PSD95 volume within microglial CD68-positive structure (Fig. S8, arrowheads). Together, these finding suggested that 72 h SD affects synaptic pruning via downregulates the capacity of microglia phagocytosis of postsynaptic materials.

6.3. Reduced expression of microglia-specific receptors after SD in adolescent mice

Our results demonstrated that increased spine density in sleep-

Table 1
Morphometric analysis of microglia in the molecular layer of the DG.

	Parameters					
	Adolescent			Adult		
	HC (n = 33 cells)	BP (n = 39 cells)	SD (n = 37 cells)	HC (n = 32 cells)	BP (n = 31 cells)	SD (n = 28 cells)
Bifurcation nodes	21.76 ± 0.84	23.05 ± 0.73	21.73 ± 0.90	34.22 ± 1.43	32.87 ± 1.58	21.86 ± 1.11 $^{***,\ \#\#\#}$
Terminal endings	28.27 ± 0.94	30.03 ± 0.85	28.81 ± 0.98	42.16 ± 1.51	32.87 ± 1.65	28.75 ± 28.75 $^{***,\ \#\#\#}$
Primary processes	5.91 ± 0.27	6.10 ± 0.26	6.46 ± 0.22	6.56 ± 0.31	6.74 ± 0.25	6.25 ± 0.34
Total process length (μm)	396.26 ± 11.61	402.43 ± 10.15	412.27 ± 13.51	555.82 ± 21.41	513.14 ± 16.98	400.15 ± 16.39 $^{***,\ \#\#\#}$

Microglia were collected from 4 to 6 mice per group. Results are the mean ± SEM. HC, home cage; BP, big platform and SD, sleep deprivation. # indicates a significant difference between BP and SD, $p < .05$; *** and ###, respectively, indicate a significant difference between HC and SD or between BP and SD, $p < .001$.

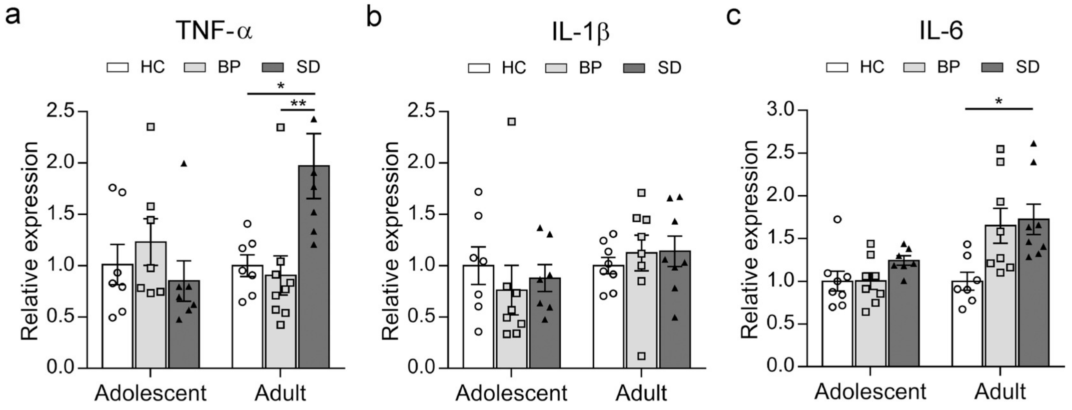

Fig. 5. mRNA expression of proinflammatory cytokines. mRNA levels of proinflammatory cytokines including TNF-α (a), IL-1β (b) and IL-6 (c). HC, home cage; BP, big platform and SD, sleep deprivation. $n = 7–9$ mice per group. Asterisks indicate significant differences between groups ($* p < .05$; $** p < .01$).

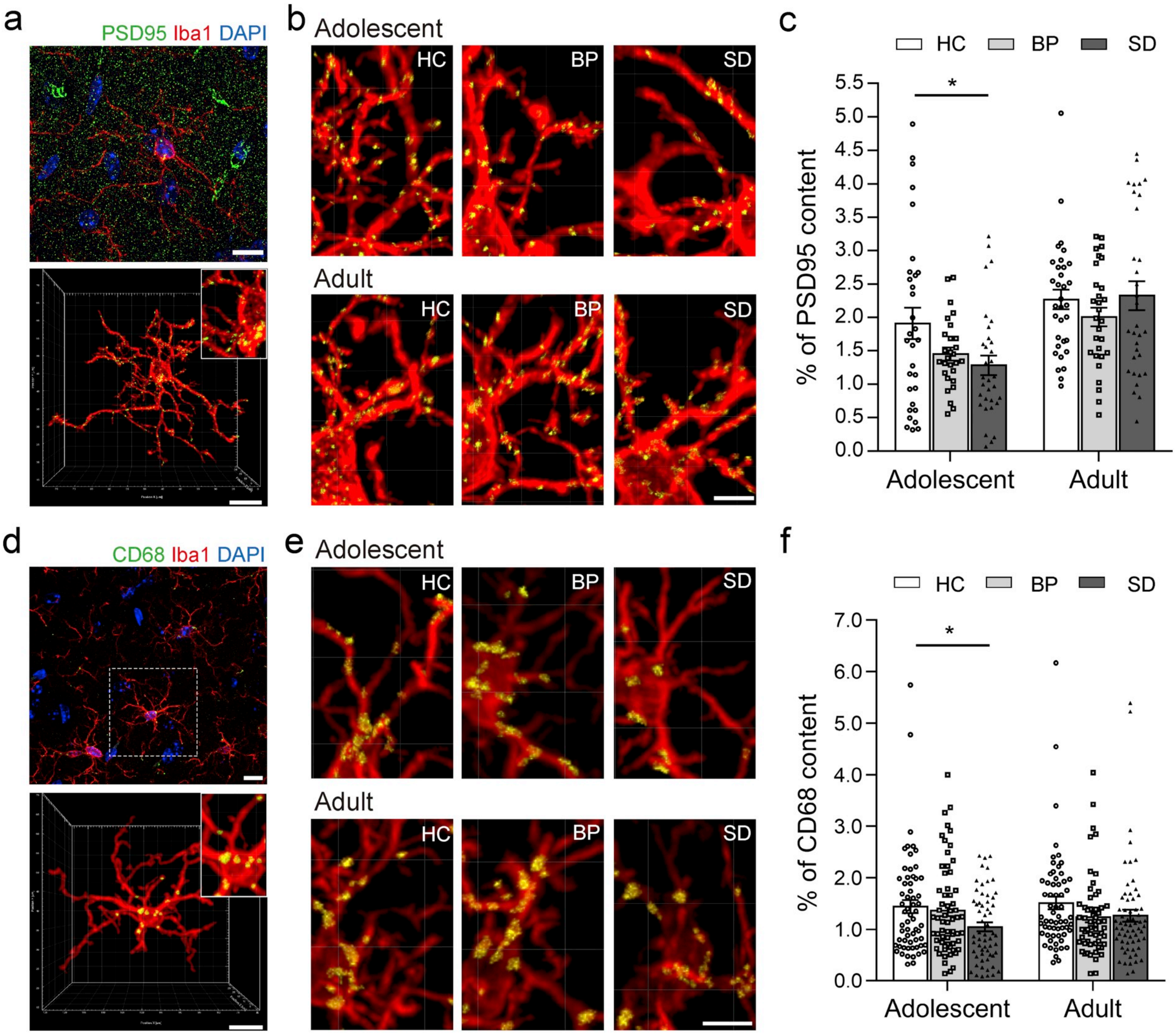

Fig. 6. Quantification of PSD95-positive puncta and a lysosomal marker within the volume of microglia. PSD95 and Iba1 were double labeled, and the volume of internalized PSD95 puncta within each of the microglia was acquired from 3D surface rendering images (a-c). Scale bar: 10 μm in a, 3 μm in c. The relative volume of PSD95 puncta within each of the microglia was measured (d). n = 28–33 cells from 3 mice per group. CD68 and Iba1 were double labeled, and the volume of CD68-positive signal within each of the microglia was acquired from 3D surface rendering images (e-g). Scale bar: 10 μm in e, 3 μm in g. The relative volume of CD68-positive signals within each of the microglia was measured (h). HC, home cage; BP, big platform and SD, sleep deprivation. $n = 58–61$ cells from 3 mice per group. Asterisks indicate significant differences between groups ($* p < .05$).

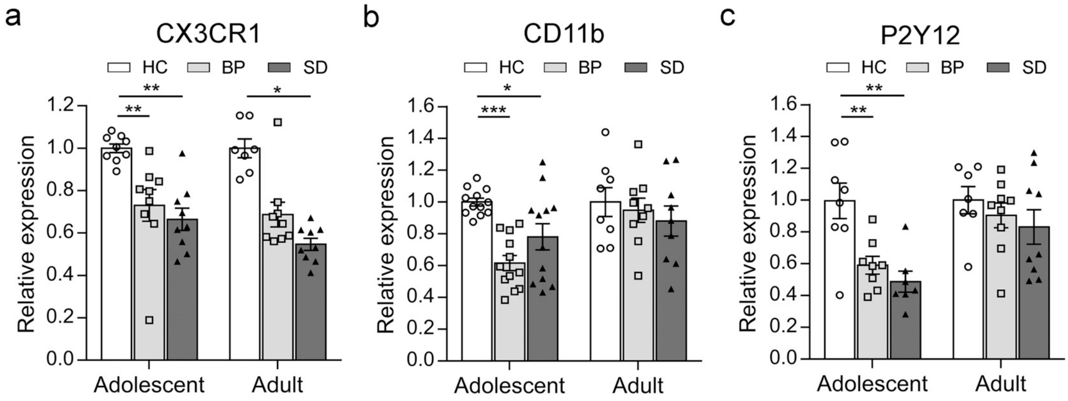

Fig. 7. mRNA expression of microglia-specific receptors. mRNA levels of CX3CR1 (a), CD11b (b) and P2Y12 (c) were measured by RT-PCR. HC, home cage; BP, big platform and SD, sleep deprivation. n = 7–9 mice per group. Asterisks indicate significant differences between groups ($* p < .05$; $** p < .01$).

deprived adolescent mice is attributable to the reduction of microglial phagocytosis. Several microglia-specific pathways are required in the process of synaptic elimination, including the fractalkine (CX3CL1)/fractalkine receptor (CX3CR1) system and classical complement signaling (Paolicelli et al., 2011; Schafer et al., 2012; Zhan et al., 2014). Accordingly, we examined the mRNA levels of CX3CR1 and CD11b, a subunit of the complement component C3 receptor (C3R) heterodimer, in the hippocampus. Significant decreases in the levels of both molecules were found in the SD and BP groups compared to the controls in adolescent mice (Fig. 7 a and b). In adult mice, downregulated expression of CX3CR1 was also noticed in the SD group (Fig. 7a). Intriguingly, the mRNA level of the $G_{i/o}$-coupled purinergic receptor P2Y12, which modulates the microglial response to early neuronal injury and neuronal activity by binding with adenosine diphosphate (Haynes et al., 2006; Sipe et al., 2016), was significantly decreased in the SD and BP groups in adolescent mice but comparable among the three groups in adult mice (Fig. 7c).

Aside from the receptors on microglia, the "eat-me" signals attached to the to-be-pruned synapse are also necessary for proper synaptic elimination. We thus examined the mRNA levels of CX3CL1 and the complement molecules C1qa and C3, which serve as opsonins to trigger C3R-dependent phagocytosis, in the hippocampal tissue. However, no difference was observed among the three groups at either age (Fig. S9). These results indicated that 72 h SD disrupted molecular pathways involved in microglia-mediated pruning in adolescents by downregulating the expression of essential receptors on microglia rather than their binding partners on neurons.

7. Impact of 72 h SD on astrocytes

7.1. *The density and phagocytic markers of astrocytes were not altered by SD in both adolescent and adult mice*

Besides microglia, we wondered if astrocytes also play a role in the neuronal changes after sleep deprivation. Astrocytes react to stimuli and show cellular hypertrophy and increased expression of glial fibrillary acidicnprotein (GFAP) (Sofroniew and Vinters, 2010; Wilhelmsson et al., 2006). We thus examined the occupancy of GFAP expression as an index of astrocyte reactivity (Fig. S10a). Our results showed that the levels of GFAP within the molecular layer of DG were comparable between three treatment groups in both adolescent and adult mice (Fig. S10b), suggesting that astrocytic reactivity may not be altered by SD.

We also examined the mRNA levels of Mertk (Mer tyrosine kinase) and MEGF10 (Multiple EGF like domains 10), in the hippocampus. Mertk and MEGF10 are involved in the astrocytic phagocytosis of synapses (Bellesi et al., 2017; Chung et al., 2013). Our results showed no difference between HC, BP and SD groups in both adolescent and adult mice (Fig. S11 a and b), suggesting that the features of astrocytic phagocytosis in the hippocampus are not significantly altered by our experimental manipulations.

8. Discussion

The current study evaluated the effects of 72 h SD on microglial reaction in hippocampus of both adolescent and adult mice. Our results revealed that 72 h SD increases excitatory synapses in DG granule cells in adolescent mice, which indicates impaired synaptic pruning. Furthermore, within the hippocampal microglia of adolescent SD mice, engulfment of postsynaptic materials and lysosomal content were reduced, which could be attributed by the decrease of microglia-specific receptors involved in synapse elimination. On the other hand, microglia-associated inflammatory reactions were significant in adult SD mice. Notably, although SD could induce age-dependent microglial reactions, similar defects in short-term recognition memory are manifested.

Our findings demonstrated that microglia-mediated synaptic pruning is more sensitive to SD in adolescent than adult brains, while the microglia-associated inflammatory reactions are more significant in adult SD mice. It has been shown that microglia-mediated synaptic pruning is predominant in the developing brain (Schafer et al., 2012). A previous study also showed that sleep-mediated synaptic turnover is relative prominent in young than adult animals (Maret et al., 2011). Our current work showed reduced microglial phagocytic activity in adolescent mice after SD. Although the link between sleep insufficiency or extended wakefulness and reduction of microglial receptors is still not clear, our finding suggested that microglia is a player that prunes the excess small and weak synapses during sleep in the developing brain. These supernumerary small and weak synapses (immature ones) might restrict the capability of subsequent learning and memory by preventing the normal strengthening of synaptic connection (Zhan et al., 2014), and consequently result in defect in the NORT. On the other hand, our results showed greater expression levels of IL-6 and TNF-α in adult SD mice. Both IL-6 and TNF-α have been implicated in disrupting learning and memory (Donzis and Tronson, 2014; Khairova et al., 2009) via modulating the compositions of AMPA receptors and decreasing excitatory synaptic strength (Lewitus et al., 2016), inhibiting long-term potentiation (Cunningham et al., 1996; Tancredi et al., 2000) or regulating the expression of neurotropic factors (Golan et al., 2004). Greater microglia-associated inflammatory reactions in adult SD mice may therefore lead to synaptic defects and impaired performance in the NORT.

Another interesting finding in the current study is the expression of neuronal activity marker, c-fos. Sleep deprivation *pre es* might cause increased expression of immediate early genes in certain brain regions participating sleep-wake behaviors (Cirelli et al., 1995; Semba et al., 2001). In the present study, we found no statistical difference in the basal level of c-fos in the dentate gyrus among the three treatment groups in either adolescent or adult mice, suggesting that the elevations of c-fos positive cells after SD were induced by the behavioral tests. In adolescent SD mice, the microglia-mediated synaptic pruning was impaired, leaving excess small and weak synapses in the DG which may result in an increased connectivity with decreased specificity represented by massive c-fos expression after the behavioral tests. On the other hand, in adult mice, although we did not observe significant difference in microglia-mediated synaptic pruning between HC, BP and SD groups, greater mRNA levels of proinflammatory cytokine, including TNF-α and IL-6 were noted only in SD group. Elevated proinflammatory cytokines levels may trigger "weak excitotoxicity" with increased extracellular glutamate and activity-induced Arc expression in neuroinflammatory models (Mandolesi et al., 2015). Increased TNF-α and IL-6 levels may account for the increased c-fos expression in adult SD mice. In order to achieve successful pattern separation during memory acquisition, only a relative small fraction of the granule cells in the DG should be activated in the event, which is known as sparse coding (Stefanelli et al., 2016). Therefore the massive increase in the numbers of activated neuron during the NORT in sleep-deprived mice, either owing to the defective synaptic pruning in adolescent SD group or the pro-inflammatory condition in adult SD group, may exert negative effects on pattern separation, resulting in impaired memory performance. In the BP groups, however, the numbers of DG c-fos-positive cells were elevated yet not surpassed a critical level, animals seemed performed well in the NORT.

In adolescent SD mice, impaired microglia-mediated synaptic pruning was noted. It is in line with previous reports. Two-photon live imaging revealed that in adolescent mice, the elimination rate of dendritic spines was significantly higher during sleep than during wake (Yang and Gan, 2012). In young but not in adult mice, a greater spine loss during sleep than during wake, with a net increase after sleep deprivation, has been found (Maret et al., 2011). Pruning of supernumerary synapses is particularly active during adolescence in response to overproduction of axons and synapses during early puberty (Arain et al., 2013; Casey et al., 2008; Petanjek et al., 2011), and this pruning is probably achieved during sleep, when brain activity is disconnected from external inputs (Tononi and Cirelli, 2014). Our findings resonate with the above studies and suggest microglia is a major player mediating this pruning process in adolescent brain.

Recently, evidence of microglia "trogocytose" synaptic material in the developing hippocampus has been assessed (Weinhard et al., 2018). Instead of entirely stripping and/or phagocytosing the whole pre- and postsynapse structure, microglia were found to nibble (trogo-) small fragment of presynaptic bouton, thus inhibit subsequent synapse formation. In the work of Weinhard et al. (2018), the neurons were labeled with cytoplasmic markers, and the microglia-neuron contacts (axon or dendrite) were captured by confocal microscopy then processed and 3-D reconstructed using focused ion beam scanning electron microscopy. The specificities of microglia-neuron interaction can be analyzed in 3D at an ultrastructural level. Nevertheless, although the detailed underlying mechanisms including direct phagocytosis of pre- and postsynaptic materials (Filipello et al., 2018; Kim et al., 2017; Paolicelli et al., 2011; Schafer et al., 2012; Sipe et al., 2016 and present study) or trogocytosis of presynaptic strictures (Weinhard et al., 2018), are not yet clear, microglia-mediated synaptic removal is essential to reduce supernumerary neuronal connections in the developing nervous system.

Microglia intimately contact pre- and postsynaptic elements express receptors that that can be stimulated by neurotransmitters, neuropeptides and neuromodulators; microglia thus response rapidly to neuronal activity (Kettenmann et al., 2011). In the visual system of mice, for example, the motility of microglial processes is modulated by sensory experience (Tremblay et al., 2010). There, microglia might play a key role by mediating the process of neural activity-dependent synaptic pruning. Manipulation of neural activity by TTX or forskolin also affects the phagocytic action of microglia, making them prone to phagocytose the weaker inputs via complement-dependent pathways (Schafer et al., 2012). In addition to the participating roles of CX3CL1/CX3CR1 signaling and the classical complement system, a microglia-specific purinergic receptor, P2Y12, has been shown to modulate this process. With either pharmacological or genetic manipulation of P2Y12 signaling, microglia exhibit basal ramification and phagocytosis of synaptic elements in response to monocular deprivation, which, in turn, leads to abrogation of ocular dominance shift (Sipe et al., 2016). The subsequent sleep, similarly, plays an essential role in activity-dependent plasticity during the critical period in the binocular visual cortex of cats (Frank et al., 2001). Together, distinct neuronal activities during sleep might regulate microglia-dependent phagocytosis of synapses; the underlying mechanism merits further investigation.

In our study, a subtle reduction in dendritic complexity was observed after 72 h experimental manipulations in adolescent but not adult mice, indicating that the structural plasticity of dendrites is particularly sensitive in adolescents relative to adults. The present study is not conclusive about microglia dysfunction participants in the reduction of dendritic complexity after acute sleep deprivation; howbeit, the absence of microglial CX3CR1 is suggested to lead to delayed maturation of adult-born hippocampal granule neurons with altered dendritic morphologies in the DG (Bolos et al., 2018; Xiao et al., 2015). Thus, it is worth noticed that acute sleep deprivation may also exert long-term effects on the developing brain by leaving a less mature phenotype of adult-born neurons and should be addressed in the future.

In the present study, the proinflammatory effect of SD is evident on adult but not adolescent mice. Age-dependent discrepancies of microglial activation and regulation have been demonstrated in the model of systemic inflammation, in which higher expression of proinflammatory genes in the middle-aged (9–10 months old) then young (2 months old) mice after acute bacterial lipopolysaccharide challenge was noted (Nikodemova et al., 2016). Our study provided the first evidence of age-dependent microglial reactions between adolescent (5 weeks old) and adult (~12 weeks old) mice. There might be intrinsic factors for microglia development and responses as well as extrinsic or environmental differences between adolescent and adult brains under normal and pathogenic conditions. It is important to clarify the underlying mechanisms of these changes. During neural development, various types of neurons and glial cells, including microglia, are engaged in the processes of neural network maturation; including synaptic strengthening or depression, apoptosis, myelination, axonal and dendritic sculpting as well as synaptic pruning. In adolescent mice, these maturation processes might be affected, to different degree, by SD. While neural network is matured, microglia may than be more active in other functional aspects, such as surveying the adjacent environment and reacting to local or global stimuli.

We also observed a downregulation of CX3CR1 mRNA level after SD in adult mice. The CX3CL1/CX3CR1 signal is involved in neuroprotection under the conditions of inflammation or excitotoxicity (Arnoux and Audinat, 2015; Sheridan and Murphy, 2013). Disruption of this signal may provoke microglia undergo transition from the normal resting state to activation or exaggerated response to stimulus (Kettenmann et al., 2011). In addition to its role as a constitutional brake signal to microglia activation, CX3CL1 is expressed abundantly in mature neurons of the adult hippocampus and in response to spatial learning and LTP induction, along with regulation of glutamatergic neuroplasticity through enhanced inhibitory tone of GABAergic transmission (Sheridan et al., 2014), thus modulated memory-associated synaptic plasticity directly. Further studies are needed to explore the possible role of microglia in synaptic homeostasis in the brain during sleep, since evidence of microglia that execute as a monitor of synaptic function has been observed in both developing and adult brains (Wake et al., 2009).

It is a critical issue to resolve the impacts of SD, SD-associated stress and both in our model. However, it is difficult to distinguish the consequences of these factors. Prolonged sleep loss could cause mild activation of the HPA axis and has been demonstrated in other experimental sleep deprivation paradigm (Colavito et al., 2013; Meerlo et al., 2002) and human sleep apnea and insomniac patient (Alkadhi et al., 2013), since HPA axis activity is required for keeping animals awake (Meerlo et al., 2008). Therefore, we believe that in our SD model, stress is inevitable. Besides sleep deprivation, stress might come from the environment. In our study, the BP group was designed to control the stressful impact from the contextual change of environment. Ideally, mice in the BP group were situated in a new environment while sufficient sleep was allowed. However, it might not be the case. Previous studies on adult rats reported that paradoxical sleep in BP group was suppressed significantly compared with those in the baseline or control group (Machado et al., 2004; Ravassard et al., 2009). The same phenomenon was also observed in mice model employed the similar design, with > 2-fold of paradoxical sleep rebound after 24 h staying on the big platform compared with control baseline (Kitahama and Valatx, 1980). As social animals, rodents tend to stay on the same platform, which is not large enough for accommodation of animals under relaxation state of sleep, and awakened each other constantly (Machado et al., 2004). Therefore, some microglial changes occurred in SD group were also observed in BP group, particularly in adolescent mice. We thus suggested that microglia homeostasis, particularly in adolescent, is sensitive in response to sleep insufficiency and associated stress. Perturbation of microglia homeostasis may result in hippocampal neuronal or behavioral alternations if sufficient time is giving, which required further studies. To note, previous studies indicated that chronic exposure to stress or stress hormone elevation often result in a reduction rather than increase of spine density in hippocampus of both adolescent (Clarke et al., 2019; Pillai et al., 2012) and adult animals (Pawlak et al., 2005). Although activation of stress system is accompanied with sleep deprivation paradigm, it is unlikely the cause of increased dendritic spines and PSD95 puncta in SD adolescent mice.

To our knowledge, the current study is the first to evaluate the reactions of microglia after SD in both adolescent and adult hippocampus. Our findings suggested that, given the fact that adolescence is a critical time window for the maturation of neural circuits, adolescent sleep insufficiency might impair the maturation of neural circuits related to learning and/or memory by interfering with the capacity for microglial phagocytosis, elaboration of dendritic structure and the pruning process of dendritic spines. On the other hand, the intensified microglia activation and elevated proinflammatory cytokines are noted in adult SD mice, suggesting that sleep insufficiency posits the adult brain under the threat of neuroinflammation and cognitive impairment. Sufficient sleep is indispensable for both adolescent and adult subjects.

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Financial interest

None.

Non-financial interest

None.

Conflict of interest

All authors declared no conflict of interest neither financially nor non-financially.

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