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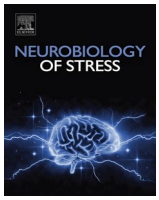

Dysfunctional synaptic pruning by microglia correlates with cognitive impairment in sleep-deprived mice: Involvement of CX3CR1 signaling

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ABSTRACT

Microglia are involved in sleep/wake cycles and the response to sleep loss. Synaptic pruning by microglia is necessary for central nervous system circuit refinement and contributes to cognitive function. Here, we investigated whether and how microglia-mediated synaptic pruning may be involved in cognitive deficits induced by sleep deprivation in mice. Mice were deprived of sleep by leaving them in a spontaneously rotating rod for 72 h, after which their cognitive function was assessed using an object location test, Y maze, and novel object recognition test. Sleep deprivation lowered the discrimination index for familiar locations in the object location test and Y maze. Microglial morphology was assessed using immunostaining Iba1, while microglia-mediated synaptic pruning was examined based on immunostaining PSD95, CD68, and Iba1. Sleep deprivation also activated microglial cells in the hippocampus, as reflected in bigger soma as well as fewer and shorter branches than normal sleep. Sleep deprivation downregulated phagocytic markers and internalization of postsynaptic protein 95 (PSD95), suggesting impaired synaptic pruning. CX3C motif chemokine receptor 1 (CX3CR1) signaling was detected in *in vitro* experiments. Sleep deprivation also downregulated CX3CR1. Activation of CX3CR1 signaling increased phagocytosis activity of BV2 microglia *in vitro*. Sleep deprivation dysregulates microglial CX3CR1 signaling and inhibits synaptic pruning, contributing to associated cognitive deficits. These findings identify CX3CR1-dependent synaptic pruning as a potential therapeutic target in which sleep deprivation causes recognition impairments.

1. Introduction

The prevalence of inadequate sleep among adolescents has been increasing worldwide (Galland et al., 2018). Sleep deprivation has been linked to memory decline and lower performance on learning tests and memory tasks (Kopasz et al., 2010; Lam et al., 2011; Leng et al., 2017). Sleep disturbances may also exacerbate cognitive impairment in aging processes (Wang and Holtzman, 2020). Synaptic pruning during sleep for the establishment of stable neuronal circuits is important for cognitive function (Tononi and Cirelli, 2014; Voss et al., 2017). Synaptic plasticity is essential for cognitive function during sleep (Wang et al., 2011). Defects in synaptic circuits because of sleep deficiency impair cognitive performance (Wang et al., 2020; Zhan et al., 2014). Given the effect of sleep deprivation on cognition, elucidating the cellular and molecular pathways affected by sleep deprivation is clearly of social and clinical importance.

Microglia are key drivers of synaptic plasticity in an activity-dependent manner during sleep-wake cycles (Hristovska et al., 2022). Synaptic pruning by microglia, in which "superfluous" synaptic connections are eliminated, is vital for synaptic plasticity and the development of neural networks (Paolicelli et al., 2011; Parkhurst et al., 2013; Schafer et al., 2012). Microglia can contact synapses or respond to signals released from the neuronal cell, for which they have lysosomal and movable activities (Augusto-Oliveira and Verkhratsky, 2021; Wake et al., 2009). Synaptic pruning depends mainly on the level of neuronal activities, and the variations are correlated to the awake and sleep states (Schafer et al., 2012). Accordingly, microglial processes sense neuronal activities with dynamic changes during the sleep-wake cycles (Stowell

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et al., 2019). When mice are awake, microglial processes show relatively low motility (Liu et al., 2019; Stowell et al., 2019), which increases during sleep since microglia monitor their surroundings and prune dendritic spines and filopodia (Choudhury et al., 2020; Kettenmann et al., 2013). Sleep is fundamental for learning and memory, which is considered at least in part to occur through microglial synaptic pruning (Corsi et al., 2022). On the other hand, sleep deprivation may cause dysfunctional neuron-glia interplay and synaptic pruning (Bellesi et al., 2017; Tuan and Lee, 2019; Wadhwa et al., 2017). Thus, synaptic pruning by microglia is an important facet for the pathophysiology of sleep.

Microglia-mediated synaptic pruning depends mainly on membrane molecules and surface receptors, such as C1q, CX3CR1, and TREM2, which are crucial to finding, selecting, and engulfing synapses (Kurematsu et al., 2022; Lan et al., 2022; Miyamoto et al., 2013; Wang et al., 2020). The CX3C motif chemokine fractalkine receptor (CX3CR1), exclusively expressed on microglia in the central nervous system (CNS), is an eat-me signal-related protein and is responsible for synapse elimination (Lee and Chung, 2019; Paolicelli et al., 2011). CX3CR1 regulates actin rearrangement and migration of microglia, which assist in synaptic remodeling (Ball et al., 2022; Kozareva et al., 2019; Lee and Chung, 2019; Paolicelli et al., 2011). Downregulation of CX3CR1 alters the synaptic pruning of microglia and increases spines in the dentate gyrus (Fernández de Cossío et al., 2017). In mice lacking CX3CR1, increased spines and immature synapses were found in the developing brain (Fernández de Cossío et al., 2017; Paolicelli et al., 2011). CX3CR1 in microglia appears to be upregulated during sleep, while it is downregulated in sleep-deprived mice (Tuan and Lee, 2019). However, the role of microglia-mediated synaptic pruning in sleep deprivation-induced cognitive deficits needs to be explored.

With this in mind, we hypothesize that dysregulation of CX3CR1-mediated synaptic pruning in sleep deprivation is coupled with cognitive deficits. Here, we used a mouse model of sleep deprivation to examine whether and how sleep deprivation contributes to cognitive deficits. We focused on the potential involvement of CX3CR1-mediated synaptic pruning by microglia. These findings provide the first evidence to identify microglial CX3CR1 signaling as a therapeutic target in the treatment of cognitive deficits induced by sleep deprivation.

2. Materials and methods

2.1. Animals

Adolescent male C57BL/6J mice (4 weeks old, 15–16 g) were purchased from Chengdu Dossy Experimental Animal Co. Ltd. (Chengdu, China). Throughout the study, mice were housed at 23 ± 1 °C in cages on a 12-h light/dark cycle, with lights on at 8:00 a.m. They had ad libitum access to food and water. Mice were allowed to acclimate for one week, then they were assigned to groups based on weight and subjected to experiments as described below.

All animal procedures were approved by the Ethics Committee for Animal Experimentation of the University of Electronic Science and Technology of China and conducted in strict accordance with the US National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (8th edition, revised 2010).

2.2. Sleep deprivation

Mice were allowed to acclimate for three days to a spontaneous rotating rod apparatus (Automated Sleep Deprivation System, Shanghai XinRuan Information Technology, Shanghai, China), and then deprived of sleep for 72 h continuously. Sleep deprivation started at the beginning of the dark phase, and the standard light/dark cycle was maintained. Control group mice were placed in cages similar to those containing the rotating rod and allowed to sleep undisturbed. All animals had ad libitum access to food and water throughout the experiment.

2.3. Behavioral testing

2.3.1. Open field test (OFT)

The OFT was adopted to evaluate the general locomotor activities and exploratory behaviors of the mice. The test was conducted in a cubic box with sides of 50 cm. Mice were placed in the center of the box and allowed to explore the area for 5 min. Several parameters such as distance traveled and the percentage of time spent in the central area were calculated using OFT100 software (Taimeng Tech, Chengdu, China). The operation area was cleaned between animals with 75% ethanol to eliminate odor cues.

2.3.2. Object location test (OLT) and novel object recognition test (NORT)

The OLT and NORT were used to assess learning and memory under controlled environmental conditions as described in a previous study (Chunchai et al., 2019). Tests were conducted in a white-painted square pool with a base of 70 × 70 cm^2 and a height of 50 cm. A camera was placed over the box for assessment. During the training phase, each mouse was placed in the box and allowed to freely explore two similar objects for 10 min. Tests were conducted after the training phase.

In the OLT, one of the objects was moved to a new location in the box for 10 min. The discrimination index of the moved object = [time exploring moved object 1/(time exploring object 1 + time exploring object 2)] × 100%.

In the NORT, one of the objects in the detection sessions was replaced with a novel object that was similar in height and volume, but different in shape and appearance. The discrimination index of the changed object = [time exploring changed object 1/(time exploring object 1 + time exploring object 2)] × 100%.

2.3.3. Y maze

The Y maze protocol was performed as previously described (Kraeuter et al., 2019). Briefly, the Y maze apparatus was placed in a quiet room, and light intensity was held at a constant level (100 lux). It consisted of three arms (50 cm length, 27 cm height, 12 cm width) arranged at a 120° angle relative to each other. During the training, one of the arms was closed with the divider, while the other choice arm was opened. The mouse was allowed to explore the maze undisturbed for 10 min. After a 1 h interval, the mice were returned to their corresponding start arm, but all doors were open. The mouse was allowed to freely enter one of two arms for 5 min. The mouse with good spatial memory will enter the previously unexplored (novel) arm more frequently than the other arms. Following each session, the apparatus was cleaned with 75% ethanol. The discrimination index was calculated as the ratio of time spent exploring the novel arm to the total time exploring both arms in the test trials.

2.4. Immunofluorescence

Mice were deeply anesthetized with phenobarbital, perfused using 0.9% saline, and fixed in paraformaldehyde (pH 7.2). Then, the brains were carefully dissected, fixed with 4% paraformaldehyde for 24 h, and dehydrated through a gradient from 10% to 30% sugar in water for 12 h. Brain slices (30 μm) were obtained using a cryostat instrument (CM1900; Leica Microsystems, Wetzlar, Germany) and stored in phosphate-buffered saline (PBS) containing 0.02% NaN_3.

For immunofluorescence staining, the brain slice was treated with Immunol Staining Wash Buffer (5 min; catalog no. P0106C, Beyotime, Chengdu, China) for 5 min, followed by Quick Antigen Retrieval Solution for Frozen Sections (catalog no. P0090, Beyotime) for 5 min, and QuickBlock™ Blocking Buffer for Immunol Staining (catalog no. P0252, Beyotime) for 1 h. Next, the sections were incubated overnight at 4 °C with primary antibodies, washed with PBS three times, incubated with the corresponding secondary antibody, and stained with 4′,6-diamidino-2-phenylindole DAPI for imaging. Primary antibodies targeted the following proteins: Iba1 (1:400; catalog no. ab5076, Abcam), PSD95

(1:400; catalog no. GTX133091, Genetex), and CD68 (1:600; catalog no. ab125212, Abcam). The following secondary antibodies were used: donkey anti-mouse IgG (1:1000; catalog no. A21202, Invitrogen Life Technologies), donkey anti-rabbit IgG (1:1000; catalog no. A21207, Invitrogen Life Technologies), goat anti-rabbit IgG (1:1000; catalog no. A32731, Invitrogen Life Technologies), and donkey anti-goat IgG (1:500; catalog no. 705-585-003, Jackson ImmunoResearch, USA). Immunofluorescence images were obtained using a fluorescence microscope (Carl Zeiss, Jena, Germany) and analyzed using Image J (version 1.45, US National Institutes of Health, Bethesda, MD, USA).

Microglial density was determined by dividing the number of microglial cells by the total sample volume (mm^3). Microglial morphology was quantified using Image J The analysis of PSD95/CD68 within microglia was quantitated using Image J based on its correlation coefficient.

2.5. Western blotting

Mice were perfused with 0.9% normal saline. Hippocampus samples were dissected from mouse brains and immediately placed in dry ice. The total soluble protein concentration was assayed using a bicinchoninic acid kit (catalog no. P0012S, Beyotime). The protein samples were separated on 12% Tris-glycine sodium dodecyl sulfate-polyacrylamide gels and transferred to a polyvinylidene difluoride membrane (Merck Millipore, Darmstadt, Germany). The different membranes were incubated with antibodies against β-actin (1:2000; catalog no. GB11001, Servicebio, Wuhan, China), GAPDH (1:1000; catalog no. GB11002, Servicebio), TREM2 (1:1000; catalog no. ER2902-96, Huabio, Zhejiang, China), CX3CR1 (1:1000; catalog no. bs-1728R, Bioss, Peking, China), synapsin (1:600; catalog no. G05063058, Wanleibio, Shenyang, China) or PSD95 (1:400; catalog no. GTX133091, Genetex, San Antonio, USA). Then, the membranes were incubated with horseradish peroxidase-conjugated goat secondary antibodies against rabbit IgG (1:10000; catalog no. ab6721, Abcam) or mouse IgG (1:10000; catalog no. ab6789, Abcam). Signals were developed using the ECL-Plus kit (catalog no. P0018S, Beyotime). Densitometry was performed by Image J to quantify signal intensity.

2.6. Cell culture and treatments

BV2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (Gibco, USA) at 37 °C with 5% CO_2. For experiments, cells were seeded into six-well plates, and when confluence exceeded 60%, half of the wells were treated with CX3CL1 (30 ng/ml, R&D Systems), while the other half were treated with saline. The phagocytosis activity of BV2 cells that were either treated with CX3CL1 or untreated was assayed using microbeads with a diameter of 0.2 μm (Bio-Rad). Cells were incubated for 2 h with microbeads (1 μl), washed with PBS to eliminate free microbeads, and fixed onto glass slides using paraformaldehyde. Microglia were stained using an anti-Iba1 antibody (1:600; catalog no. ab5076, Abcam) for 24 h at room temperature, washed with PBS three times, and incubated with donkey anti-goat IgG (1:500; catalog no. 705-585-003, Jackson ImmunoResearch). Immunofluorescence images were obtained using a fluorescence microscope (Carl Zeiss) and analyzed using Image J.

2.7. Golgi -Cox staining

Brain tissue was fixed with 4% paraformaldehyde for 48 h. The solution on the surface of the brain was washed away with saline, and the samples were soaked with a Golgi staining kit (catalog no. ZC-G1069-1, Servicebio) for two weeks in the dark at room temperature. Next, samples were incubated with strong ammonia water (25%) for 45 min followed by acid hardening fixer (catalog no. ZC-G1069-2) for 45 min, washed with saline, dehydrated with sucrose solution (30%) for 48 h, and then sectioned to a thickness of 100 μm using a cryostat (CM1900; Leica Microsystems, Wetzlar, Germany). Samples were mounted with glycerogelatin (catalog no. G1402-30 ml, Servicebio) and imaged under fluorescence microscopes (Carl Zeiss). The number of dendritic spines in hippocampal neurons was determined using Image J.

2.8. Statistical analyses

All data were analyzed using GraphPad Prism 7.0 (GraphPad Software Inc., La Jolla, CA, USA). All data are reported as the mean ± standard error of the mean. Intergroup differences were assessed for significance using, as appropriate, unpaired Student's *t*-test, Welch's correction, or a nonparametric Mann-Whitney test. Differences associated with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Sleep deprivation compromises the cognitive function of adolescent mice

To verify the effect of sleep deprivation on cognition in mice, mice were subjected to the OLT and NORT, which assess cognition function. During the training phase, both control and sleep deprivation mice spent an equal amount of time exploring the two objects (Fig. 1a). When tested for location memory 24 h later, control mice distinguished the changed location from the familiar location to a greater extent than sleep-deprived mice (Fig. 1b). However, in the NORT, there was no significant difference in the discrimination of familiar objects between the sleep-deprived and control groups (Fig. 1c). Cognitive function was also detected by the Y maze task, which takes advantage of rodents' spontaneous exploration nature. The percentage of time spent in the novel arm was markedly decreased in the sleep-deprived mice compared with the control mice (Fig. 1d). There were no significant differences in body weight between the two groups (Fig. 1e). In the OFT, the entries of mice into the central area, exploration time and distance also showed no significant differences between the sleep deprivation and control groups (Fig. 1f). These results indicated that sleep deprivation impaired cognitive function, but not locomotor activity.

3.2. Sleep deprivation hyperactivates hippocampal microglia in adolescent mice

The density of Iba1-positive microglia and their morphometric profiles were examined within the hippocampus, as Iba1 served as a reliable marker for identifying microglia. Sleep deprivation decreased the average number of microglia in the dentate gyrus and CA1 areas of the hippocampus, but not in the CA3 area (Fig. 2a–b). Then, the morphology of microglial cells was analyzed, since it is related to their state of activation. An enlarged soma of microglia was detected following sleep deprivation in the dentate gyrus (Fig. 2c). Sleep deprivation significantly reduced the length and number of branches of microglia in the dentate gyrus and CA1, but not in the CA3 (Fig. 2d–e). These findings indicate that sleep deprivation abnormally activated microglia, particularly in the dentate gyrus and CA1.

3.3. Sleep deprivation inhibits microglia-mediated synaptic pruning

By double-labeling with PSD95 antibody and Iba1 antibody, we found decreased colocalization of PSD95 and Iba1 positive immunoreactivities in sleep deprivation mice in the dentate gyrus and CA1, but not CA3 (Fig. 3a–b). It also decreased the levels of hippocampal microglia expressing CD68 (Fig. 3c–d), a lysosomal marker whose levels reflect the strength of phagocytic activity (Schafer et al., 2012). We examined the expression of postsynaptic proteins (PSD95) and presynaptic proteins (synapsin) in the hippocampus using western blotting. Sleep-deprived mice expressed higher levels of PSD95 and synapsin than control animals (Fig. 3e–f). These findings suggest that sleep deprivation inhibited

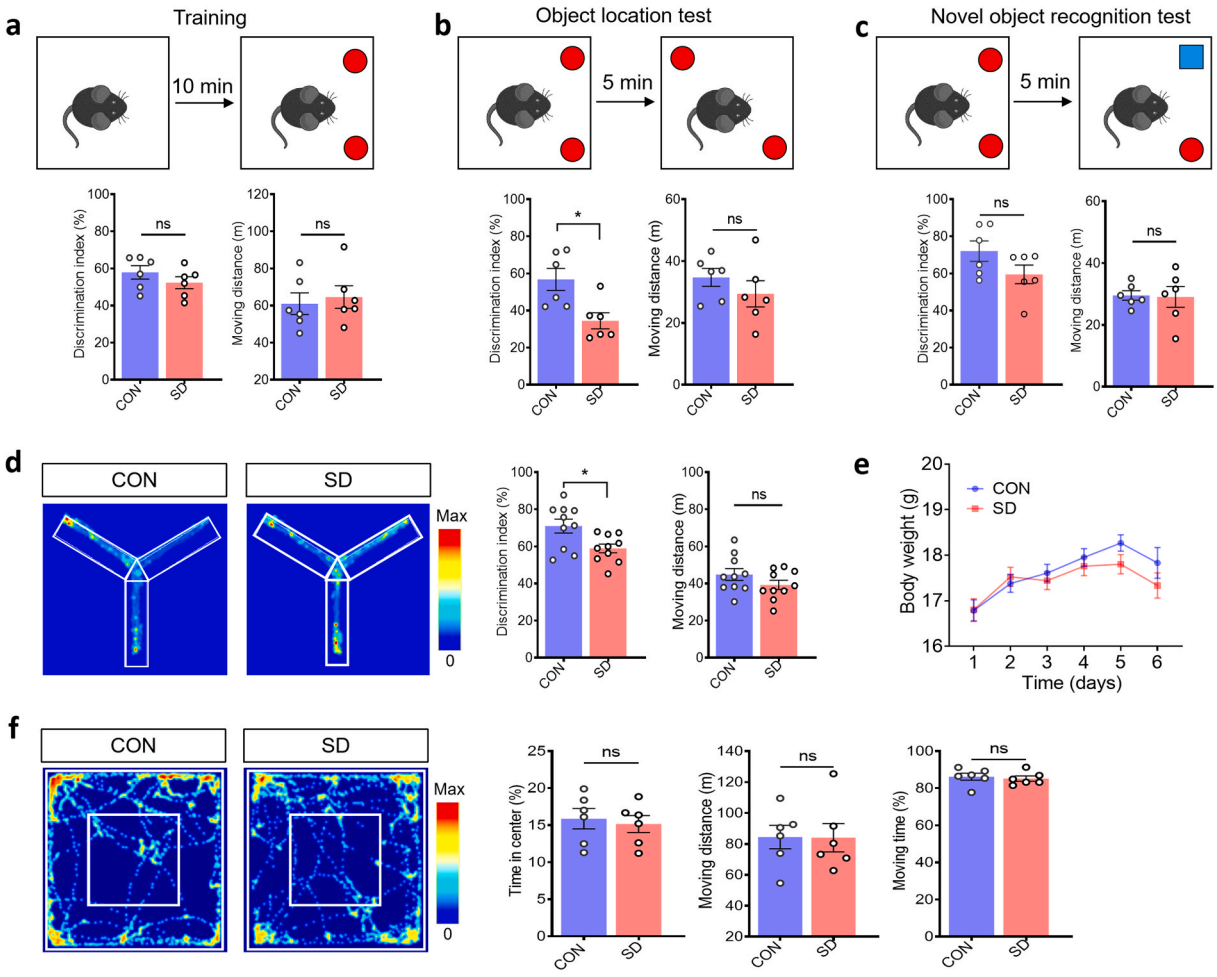

Fig. 1. Effects of sleep deprivation (SD) on cognitive function in adolescent mice. (a) In the training session, the discrimination index to two similar objects and the training distance were assessed. (b) In the object location test (OLT), the discrimination index of time spent exploring a novel location versus a familiar location and the moving distance was assessed. (c) In the novel object recognition test (NORT), the discrimination index of time spent exploring a novel object versus a familiar object and the moving distance was assessed. (d) In the Y maze, the discrimination index of time spent exploring a novel arm versus a familiar arm and the moving distance were assessed. (e) Body weight was measured daily during the casting session. (f) In the open field test (OFT), the time spent in the center, distance traveled, and time spent moving were assessed. Data are mean ± standard error of the mean (n = 6 animals per condition). *P < 0.05, **P < 0.01, ***P < 0.005.

synaptic pruning by microglia. Indeed, sleep deprivation increased the density of dendritic spines, based on Golgi-Cox staining (Fig. 3g). The change in spine number is associated with functional changes in synaptic connectivity and behavioral changes (Liu et al., 2017). These results suggest that sleep deprivation profoundly decreases microglia-mediated synaptic pruning capacity.

3.4. Sleep deprivation acts via CX3CR1 signaling to impair synaptic pruning by microglia

We verified the pro-phagocytotic effect of CX3CR1 using cultures of BV2 microglia (Fig. 4a). CX3CL1, which induced the expression of CX3CR1, reduced the branch length in BV2 cells, without affecting the cell body area (Fig. 4b–d). The internalization of BV2 microglia microspheres was also detected, when CX3CL1 was administered at different time points. Independent of time, CX3CL1 also promoted BV2 microglia to phagocytose microspheres (Fig. 4e). This result was consistent with impaired microglia-mediated synaptic pruning after sleep deprivation *in vivo*. Sleep deprivation downregulated CX3CR1 in the hippocampus of mice, but not TREM2 (Fig. 4f–g). These results suggest that CX3CR1 signaling may regulate microglia-mediated synaptic pruning in mice, and that sleep deprivation downregulates this pathway to inhibit pruning, leading to cognitive defects.

4. Discussion

During sleep, microglia prune synapses to consolidate neuronal circuits. Our results indicate that dysregulation of microglia-mediated synaptic pruning contributes to cognitive impairment induced by sleep deprivation. We further demonstrate that sleep deprivation may exert these effects by dampening CX3CR1-mediated signaling.

Sufficient sleep is important for brain development and cognitive performance. The OLT, Y maze and NORT are probably the most commonly used behavioral evaluations with the advantage of avoiding stimuli with strong emotional valence (Savage and Ma, 2014). In the present research, our results showed that sleep deprivation worsened the performance of mice in the OLT and Y maze, but not in the NORT. These findings indicated that cognitive impairment was seemingly not the consequence of the changes in exploratory behaviors in sleep deprivation-treated mice. Another explanation is that sleep deprivation selectively impaired spatial cognition other than recognition memory, which is reflected by OLT/Y maze tests and NORT, respectively (Jiao et al., 2022; Mumby et al., 2002). Sleep deprivation may cause memory impairment in object-location recognition that is involved in the region-specific function of the hippocampus.

Microglia sense neuronal activity and modulate synaptic activity through physical contact with their processes, which contribute to the

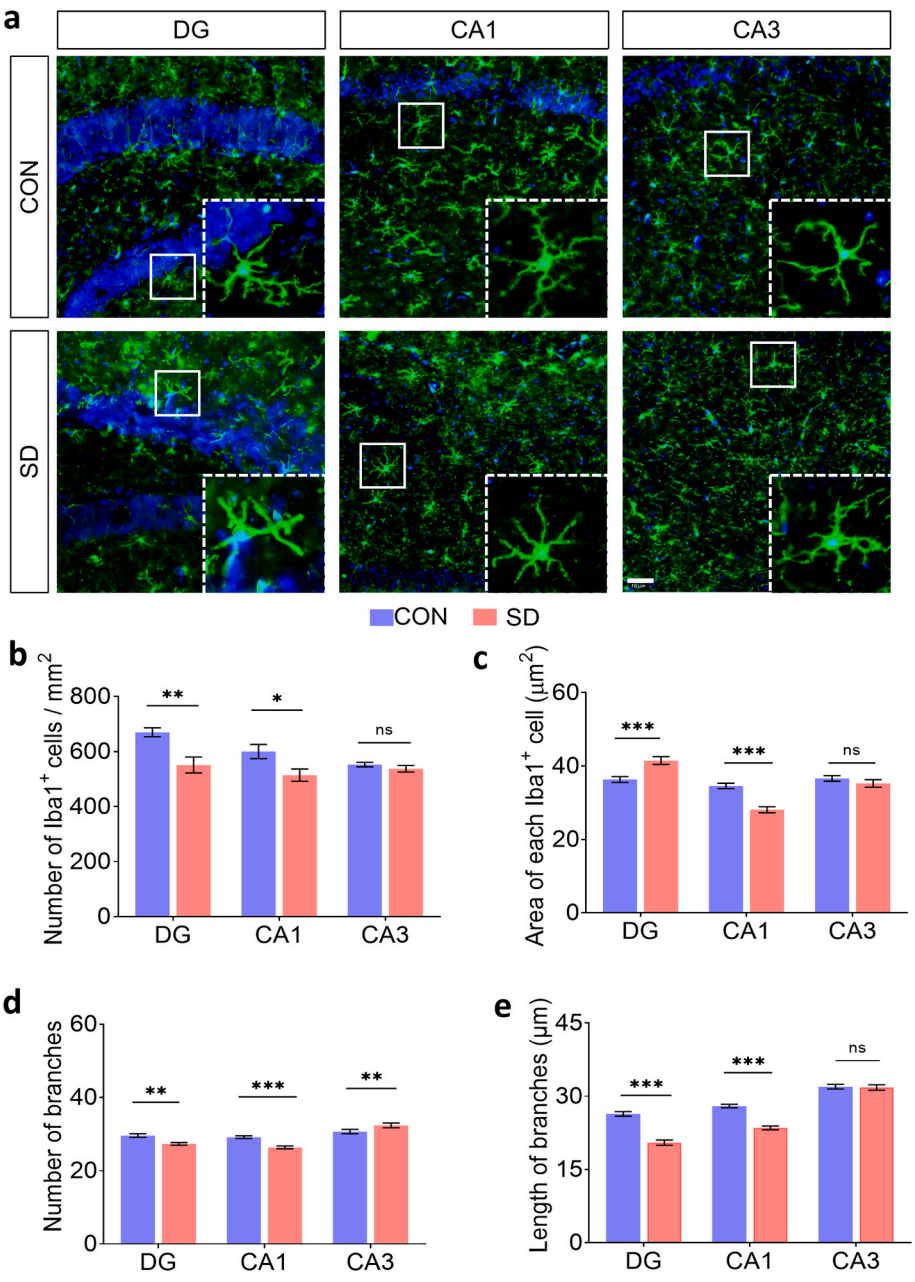

Fig. 2. Effects of sleep deprivation (SD) on the phenotype of hippocampal microglia in adolescent mice. (a) Representative morphology of hippocampal microglia in control and sleep-deprived mice. (b) Numbers of Iba1$^+$ cells in the hippocampus. (c) Area of Iba1$^+$ cells in the hippocampus. (d) Numbers of branches on Iba1$^+$ cells in the CA1, CA3, and dentate gyrus (DG) areas of the hippocampus. (e) Lengths of branches on Iba1$^+$ cells in the CA1, CA3, and DG areas. Data are the mean ± standard error of the mean. *P < 0.05, **P < 0.01, ***P < 0.005.

wiring of neuronal circuits during sleep (Deurveilher et al., 2021). We provided evidence that sleep deprivation induced the alteration of microglial phenotypes, including fewer and shorter branches as well as enlarged somatic areas. This result is consistent with the finding that the sleep/wake cycle strongly affects microglial morphology (Choudhury et al., 2021; Nakanishi et al., 2021). Interestingly, we observed that these changes were region-dependent in sleep-deprived mice, with more alterations of activated microglia occurring in the dentate gyrus and CA1 than in the CA3. Moreover, PSD95 and CD68 internalization by microglia also occurred in a region-dependent manner, concomitant with the alteration of microglial phenotypes. Within the hippocampal microglia of sleep-deprived mice, engulfment of postsynaptic materials and lysosomal content was reduced, associated with the elevation of dendritic spine numbers and immature synapses. These findings showed that sleep deprivation inhibited synaptic pruning by microglia. Sleep deprivation caused region-specific synaptic pruning in the hippocampus, supporting the theory of selective synaptic downscaling during sleep (Havekes et al., 2016). Sleep deprivation may have different effects on microglial biology required for synaptic plasticity, depending on the regional heterogeneity of microglia (Grabert et al., 2016; Hsu et al., 2003). The distinct pattern of microglial activation in the hippocampus is consistent with evidence that the dentate gyrus, CA1, and CA3 contribute distinctly to cognitive events. The dentate gyrus and CA1 region of the hippocampus are necessary for location recognition memory, while CA3 appears to be associated with nonspatial tasks (Farovik et al., 2010; Stackman et al., 2016). Although the results suggest the relevance of the cognitive changes to the microglia-mediated synaptic pruning in sleep-deprived mice, future studies are necessary to determine the causality.

The dysfunction of microglial remodeling of synaptic plasticity is associated with sleep-related cognitive deficits. Previous work has shown that C1q and TREM2 are involved in impaired microglia-mediated engulfment of postsynaptic components (Kurematsu et al., 2022; Lan et al., 2022; Miyamoto et al., 2013; Wang et al., 2020). CX3CL1/CX3CR1 signaling has been implicated in microglial pruning of dendritic spines during normal brain development (Bolós et al., 2018;

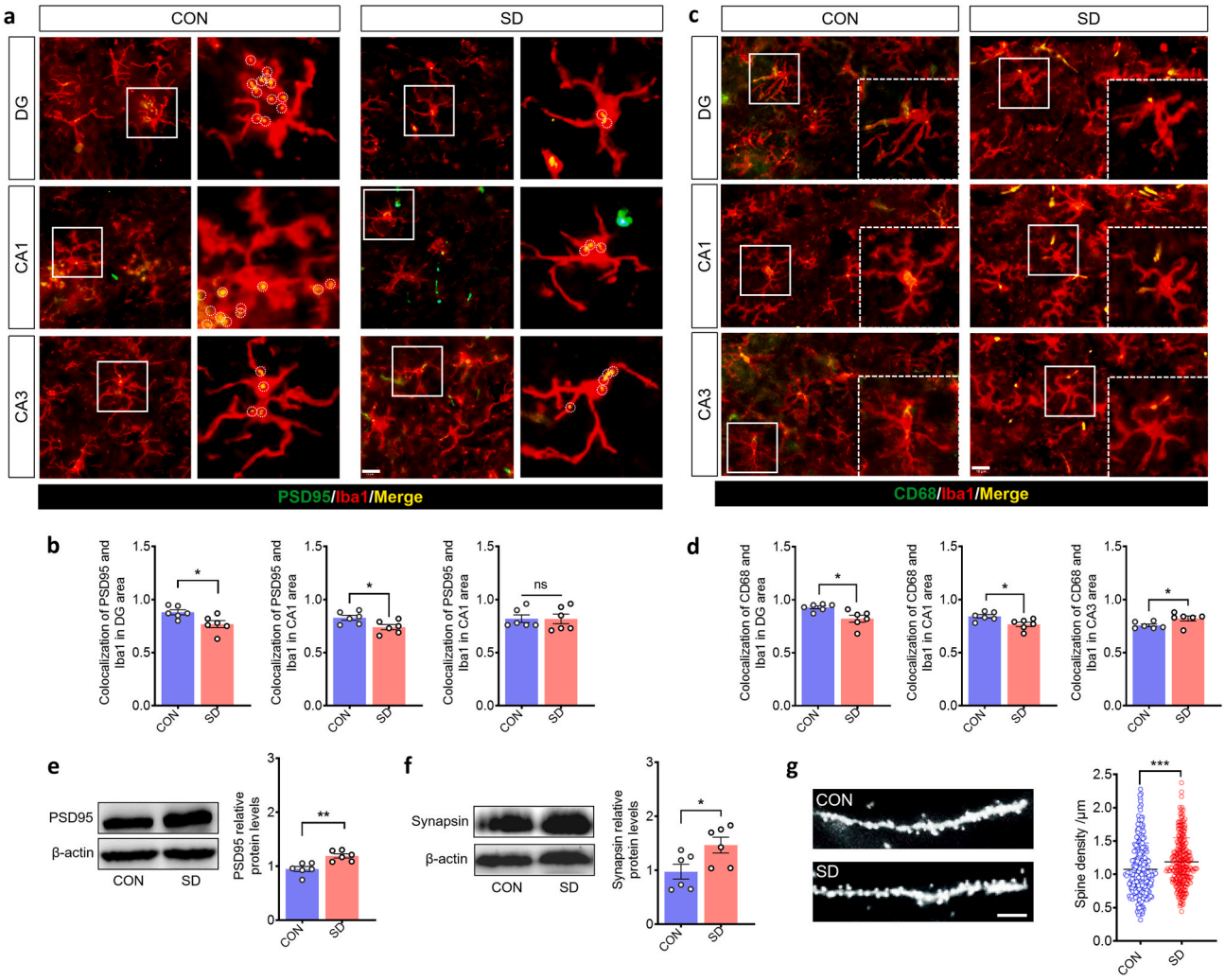

Fig. 3. Effects of sleep deprivation (SD) on microglia-mediated synaptic pruning in adolescent mice. (a) Representative image of microglial phagocytosis of synapses, based on coimmunostaining against Iba1 (red) and PSD95 (green). (b) Comparison of the correlation coefficient of anti-Iba1 and anti-PSD95 immunostaining in the dentate gyrus (DG), CA1, and CA3 areas of the hippocampus. (c) Representative image of microglial lysosomes based on coimmunostaining against Iba1 (red) and CD68 (green). (d) Comparison of the correlation coefficient of Iba1 and CD68 costaining in the dentate gyrus (DG), CA1, and CA3 areas. (e) Levels of PSD95 in the hippocampus. (f) Levels of synapsin in the hippocampus. (g) Numbers of dendritic spines per unit length. Data are the mean ± standard error of the mean. *P < 0.05, **P < 0.01, ***P < 0.005. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Paolicelli et al., 2011; Paolicelli and Gross, 2011; Ruitenberg et al., 2008). In the present study, we aimed specifically to clarify that CX3CR1 was essential for microglia and neuron communication in a sleep deprivation mouse model. Our results showed that the density of microglia was reduced, and the expression of CX3CR1, other than TREM2, was downregulated in the hippocampus of sleep-deprived mice. Using BV-2 microglial cultures, we showed that CX3CR1 signaling regulated microglia-mediated synaptic pruning. In future studies, it will be interesting to evaluate whether the enhancement of CX3CR1 relieves sleep deprivation-induced recognition impairments.

In summary, our study offers strong evidence that sleep deprivation impairs CX3CR1-mediated synaptic pruning, which may drive its harmful effects on cognition and memory. These insights may lead to effective therapeutic strategies against cognitive impairments caused by sleep disturbance.

Ethics approval and consent to participate

All animal procedures were approved by the Ethics Committee for Animal Experimentation of the University of Electronic Science and Technology of China, and conducted in strict accordance with the US National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (8th edition, revised 2010).

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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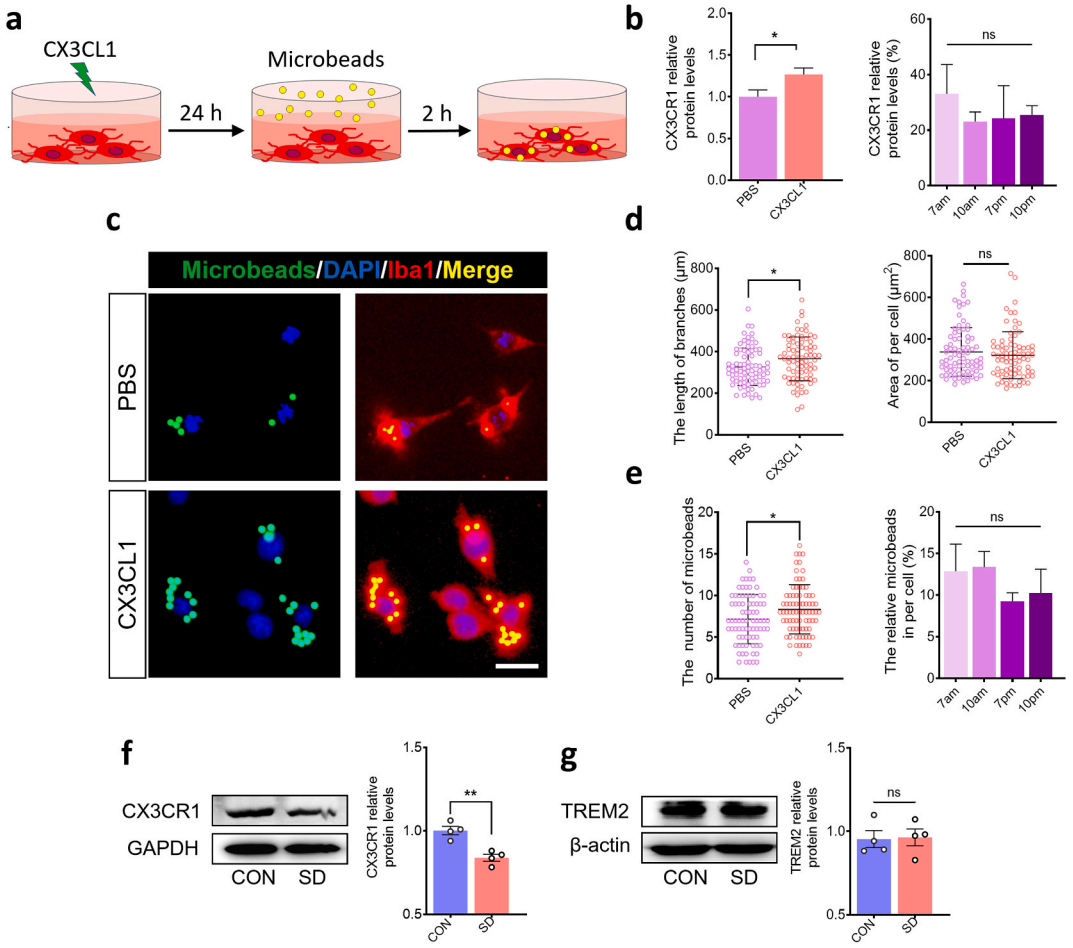

Fig. 4. Effects of sleep deprivation (SD) on CX3CR1 expression in mice and of CX3CR1 exposure on cultures of BV2 cells. (a) Schematic diagram of *vitro* experiments with BV-2 cells. (b) Alteration in the expression of CX3CR1 when CX3CL1 was administered at different timepoints. (c) Micrograph showing microglia phagocytosing fluorescent microbeads (green) after exposure to CX3CL1. Microglia were immunostained for Iba1 (red). (d) Alterations in the morphology of microglia after exposure to CX3CL1. (e) Alterations in the phagocytic activity of microglia after CX3CL1 treatment at different time points. (f) Hippocampal expression of CX3CR1 at the protein level. (g) Hippocampal expression of TREM2 at the protein level. Data are the mean ± standard error of the mean. *P < 0.05, **P < 0.01, ***P < 0.005. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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CRediT authorship contribution statement

Lu Wang: Conceptualization, Methodology, Investigation, Writing – original draft. **Hanyi Ling:** Conceptualization. **Hui He:** Methodology, Writing – review & editing. **Nan Hu:** Methodology. **Lin Xiao:** Funding acquisition, Writing – review. **Yue Zhang:** Writing – review. **Lei Xie:** Methodology. **Zili You:** Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors assert that they have no competing financial or personal interests.

Data availability

Data will be made available on request.

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Abbreviations

C1q	complement component 1q
CD68	class D scavenger receptor 68
CX3CL1	CX3C motif chemokine ligand 1
CX3CR1	CX3C motif chemokine receptor 1
DG	dentate gyrus
NORT	novel object recognition test
OFT	open field test
OLT	object location test
PBS	phosphate-buffered saline
PSD95	postsynaptic density protein 95
RT-qPCR	real-time quantitative PCR

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