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ORIGINAL PAPER

Chronic Sleep Restriction Induces Aβ Accumulation by Disrupting the Balance of Aβ Production and Clearance in Rats

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Abstract

Amyloid-β (Aβ) plays an important role in Alzheimer's disease (AD) pathogenesis, and growing evidence has shown that poor sleep quality is one of the risk factors for AD, but the mechanisms of sleep deprivation leading to AD have still not been fully demonstrated. In the present study, we used wild-type (WT) rats to determine the effects of chronic sleep restriction (CSR) on Aβ accumulation. We found that CSR-21d rats had learning and memory functional decline in the Morris water maze (MWM) test. Meanwhile, $A\beta_{42}$ deposition in the hippocampus and the prefrontal cortex was high after a 21-day sleep restriction. Moreover, compared with the control rats, CSR rats had increased expression of β-site APP-cleaving enzyme 1 (BACE1) and sAPPβ and decreased sAPPα levels in both the hippocampus and the prefrontal cortex, and the BACE1 level was positively correlated with the $A\beta_{42}$ level. Additionally, in CSR-21d rats, low-density lipoprotein receptor-related protein 1 (LRP-1) levels were low, while receptor of advanced glycation end products (RAGE) levels were high in the hippocampus and the prefrontal cortex, and these transporters were significantly correlated with $A\beta_{42}$ levels. In addition, CSR-21d rats had decreased plasma $A\beta_{42}$ levels and soluble LRP1 (sLRP1) levels compared with the control rats. Altogether, this study demonstrated that 21 days of CSR could lead to brain Aβ accumulation in WT rats. The underlying mechanisms may be related to increased Aβ production via upregulation of the BACE1 pathway and disrupted Aβ clearance affecting brain and peripheral Aβ transport.

Keywords Alzheimer's disease · Chronic sleep restriction · Amyloid-β accumulation · BACE1 · LRP-1 · RAGE

Introduction

Alzheimer's disease (AD) is an age-related, progressive neurodegenerative disease and is the main cause of dementia. There is a great deal of evidence indicating that amyloid-β (Aβ) plays an important role in the different stages of AD pathology [1, 2]. The accumulation of extracellular toxic Aβ is one of the pathological hallmarks of AD [3] and has been hypothesized to result from a production and clearance imbalance [4].

Aβ peptides are generated from amyloid-β precursor protein (APP) via ordinal cuts by β-secretase and γ-secretase [5]. The $A\beta_{40}$ peptide is the most abundant variety, while the $A\beta_{42}$ subtypes, which are associated with AD pathogenesis, are the most amyloidogenic form of the peptide [6]. β-site APP-cleaving enzyme 1 (BACE1) is the main form of β-secretase that acts as a pivotal regulator of Aβ generation [7]. At the same time, APP can be cleaved by α-secretase and γ-secretase without Aβ being produced. In addition, the cleavage of APP by α- and β-secretase respectively also generates soluble forms called sAPPα, the non-amyloidogenic peptide, and sAPPβ, the amyloidogenic peptides [8].

After generation in the brain, in the physiological state, some soluble Aβ could be degraded by catabolic enzymes and phagocytes in the interstitial fluid. In addition, most of the Aβ can be directly transported to the cerebrospinal fluid (CSF) and then go across the blood–brain barrier (BBB)

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into the peripheral venous blood through transporters, such as low-density lipoprotein receptor-related protein 1 (LRP-1). Meanwhile, receptor of advanced glycation end products (RAGE) could promote an influx of peripheral Aβ across the BBB from the peripheral venous blood to the brain [9, 10]. Plasma soluble LRP1 (sLRP1) sequestrates Aβ acting as an additional peripheral aid [11]. Soluble RAGE (sRAGE) binds Aβ and thus reduces the binding of these particles to membranous RAGE in the brain [12, 13]. After efflux from the brain into the peripheral plasma, large amounts of Aβ are cleared by the capillary beds of peripheral tissues and organs [14–16]. Researchers also found that increases in peripheral Aβ clearance could independently reduce the brain Aβ burden in rodents, which indicated the close relationship between the clearance of peripheral Aβ and the deposition of brain Aβ [17].

Sleep is an important physiological process, and a previous study stated that sleep could eliminate the toxins in the brain, including Aβ [18], which accumulates during wakefulness and neural activity [19]. Up until now, a bidirectional relationship between sleep disturbance and AD has been recognized. Previous clinical study has shown that sleep disturbance has been found in AD patients [20], and AD could cause sleep fragmentation related to higher CSF-orexin levels in patients with MCI [21]. Moreover, sleep disorder has been regarded as a kind of symptom of AD [22]. On the other hand, growing evidence has shown that poor sleep quality is one of the risk factors for AD. For example, longitudinal studies have found that, compared with the general population, subjects with sleep disorders demonstrated an increased prevalence of AD [23, 24]. Additionally, our previous study found that sleep deprivation could lead to an increase in plasma $A\beta_{40}$ and a decrease in the $A\beta_{42}/A\beta_{40}$ ratio in healthy young adults [25]. Furthermore, some studies found that more Aβ was deposited in the brain after sleep deprivation in the APPswe/PS1dE9 mouse model or wild-type (WT) mice [26, 27].

Despite these reports on the relationship between sleep and AD, the mechanisms of sleep deprivation that lead to cognitive impairment have still not fully been demonstrated. Therefore, in the present study, we used WT rats to determine the effects of chronic sleep restriction on cognitive behaviors and Aβ metabolism.

Materials and Methods

Animals

Seventy-two 10-month-old male Sprague–Dawley rats were used in this study, and these rodents were purchased from the animal experimental center of Xi'an Jiaotong University. All rats were housed with free access to normal food (Qinle, Xi'an, China) and water under a 12:12 h light: dark cycle, and the room temperature was controlled at 22 ± 1 °C. The composition of the food in this study is: water ≤ 10%, protein ≥ 18%, fat ≥ 4% and fibre ≤ 5%. All experimental protocols were approved by the committee on animal research at Xi'an Jiaotong University and complied with the Guidelines for the Care and Use of Animals.

Chronic Sleep Restriction Protocols

The experimental animals were randomly divided into three groups: the 7-day (7d) group (n = 24), 14-day (14d) group (n = 24) and 21-day (21d) group (n = 24). Each group was divided into two subgroups: the chronic sleep restriction (CSR) group (n = 12) and the control group (n = 12). Rats in the CSR group were placed in slowly rotating drums (40 cm in diameter) that deprived them of sleep by continuously rotated for 1 rev/min for 20 h (from 12:00 to 8:00 + 1 day), and the time of CSR lasted 7 days, 14 days or 21 days according to the different grouping situations. These rats had free access to food and water at any time and were allowed to sleep for 4 h at the beginning of each day (8:00–12:00) [28]. To reduce stress, rats were habituated to the activity of the drum motion initially and progressively for 7 days. For the first 3 days, rats were accustomed to the drum without any motion. Rats were then subjected to motion each day at 14:00 (15 min for 2 days and 30 min for the last 2 days) [29]. The day after the drum habituation sessions, CSR rats were exposed to the CSR as described previously [28]. Behavioral tests were carried out before the rats were sacrificed.

Control rats with normal sleep were placed in the same drum during the same time period as the CSR rats, but these control rats were allowed to have unrestricted sleep on every experimental day with the drum locked.

Morris Water Maze Test

The Morris water maze (MWM) test is widely used to evaluate spatial memory learning and recall performance in rodents [30]. On the 7th, 14th, and 21st days after CSR, 12 rats from each of the two groups, namely, the CSR group and the control group, were selected to undergo learning and memory function evaluations by this behavior test. The apparatus consisted of a round, white tank (120 cm in diameter, 50 cm in height). The tank, filled with tap water and milk powder, appeared opaque and was maintained at a temperature of 20–23 °C. A transparent cylinder placed 0.5 cm below the surface of the liquid milk was located in the middle of the southwest quadrant and was used as the escape platform. Before the training, all rats were accustomed to the apparatus and the opaque water. Over the subsequent 4 days, each rat was put into the water from four different start positions around the tank (north, northwest, east, and southeast)

and was allowed to swim until it found the invisible platform in 120 s. The total time of the tested rat to reach the platform was recorded in each trial. If a rat failed to reach the platform, the rat was guided to the platform and allowed to stay there for 30 s, so the escape latency was recorded as 120 s. At the end of training on day five, a probe test lasting 120 s was conducted to examine how well the memory of the tested rats, which had acquired the accurate position of the platform previously, was retained. The transparent platform was taken out of the water, and each rat was placed into the pool from the northeast quadrant and allowed to swim for 120 s. The frequency of passing through the location of the previous platform was recorded in a computer.

Tissue Collection and Blood Sampling

Rats were intraperitoneally anesthetized deeply with a solution including 2% pentobarbital sodium (60 mg/kg). Next, brain samples were immediately prepared according to each experimental technique. Blood samples were gathered by the cardiac puncture method in tubes with no anticoagulant and heparinized tubes, and then these samples were centrifuged at 2000 G for 15 min. Following this separation step, the plasma or serum was separated into 200 µl polypropylene tubes and kept frozen at − 80 °C for later enzyme-linked immunosorbent assays (ELISAs). All samples were hemolysis free upon visual inspection.

Western Blotting

Rats for western blotting in each group were anesthetized as described previously and were then perfused transcardially with 4 °C 0.9% pH 7.0 normal saline (NS). The hippocampus and prefrontal cortex were separated on ice once the decapitation was done. Then these tissues were subsequently stored in a container with liquid nitrogen for move and at -80 °C until further use. For western blotting, hippocampal tissues and prefrontal cortex tissues were ground in RIPA buffer (P0013B, Beyotime, Shanghai, China) dissolving protease inhibitor tablets (complete, Roche, Germany). After the supernatant was collected, BCA protein assay kits (P0010, Beyotime, Shanghai, China) were used for quantitative determination of the protein samples. A total of 40 µg of each protein sample was used for electrophoresis with 10% sulfate–polyacrylamide gels, followed by wet transfer to polyvinylidene fluoride (PVDF) membranes (Millipore). These membranes were blocked by using 5% nonfat dry milk in TBST for 1 h, washed in TBST three times and then incubated with primary antibodies at 4 °C overnight. The following primary antibodies were used: rabbit antibody anti-APP (ab32136, 1:10,000, Abcam), LRP-1 (ab92544, 1:5000, Abcam), and RAGE (ab3611, 1:1000, Abcam) primary antibodies. After the above steps, the membranes were washed, and after that, the corresponding secondary antibody (1:10,000; Abmart) was used for incubation for 1 h. The immune bands were detected by an enhanced chemiluminescence (ECL) system according to the product's specifications. β-actin antibody (L3032, 1:5000, SAB) was used as the internal reference protein. The gray value of the bands was quantified using the ImageJ software (version 1.50i, NIH, USA).

ELISAs

An ELISA was used to measure brain $A\beta_{42}$, BACE1, sAPPα and sAPPβ and plasma $A\beta_{42}$, $A\beta_{40}$, sRAGE and sLRP1. The protocol followed the manufacturer's specifications (ELISA, Yuanye, Shanghai, China), and 5 M guanidine HCl was used for tissue homogenization. The measurements were processed on a Rayto RT-6000 analyzer (Rayto, Shenzhen, China) at 450 nm, and concentrations were calculated by using standard curves. All samples were analyzed three times.

Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA of the hippocampus and prefrontal cortex was extracted from rats using Trizol reagent according to the product's specifications. Reverse transcript (RT) involved 5 µg of total RNA. The samples were subsequently subjected to first-strand cDNA synthesis using a QuantiTect Reverse Transcript Kit (#K1621, Thermo Scientific). The resulting cDNA was amplified using 2 × Sybr Green qPCR Mix (PC3302, Aidlab). PCR was performed initially at 94 °C for 3 min, followed by 40 cycles of 94 °C for 10 s and 60 °C for 34 s. The relative gene below was calculated using the $2^{-\Delta\Delta C}{}_t$ method. It can be reasonably supposed that the magnification efficiencies of the genes included in the study were equal in this $2^{-\Delta\Delta C}{}_t$ calculation method. The values were normalized against the reference gene β-actin. The primer sequences were as follows: for APP the forward was 5-AGTAGCCGAAGAGGAGGAAG-3 and the reverse was 5-CAGTGGTAGTTGTGGTAGTGG-3, for LRP-1 the forward was 5-GAGCAGGTTGTAGTC AGCA-3 and the reverse was 5-TAGGGTTTCCGATTT CCACA-3, for RAGE the forward was 5-TGAATCCTG CCTCTGAACTC-3 and the reverse was 5-TCTGACCGA AGCGTGAAG-3, for BACE1 the forward was 5-CGC TGGCTCCTGCTATGG-3 and the reverse was 5-GGT TCCTCGTCCGTCTCC-3, and for β-actin the forward was 5-CTATCGGCAATGAGCGGTTCC-3 and the reverse was 5-TGTGTTGGCATAGGTCTTTACG-3.

Immunohistochemical Staining (IHC)

Rats randomly selected from the control and CSR groups were anesthetized as previously described for immunohistochemical testing. These rats were perfused transcardially with 4 °C 0.9% pH 7.0 NS, followed by 4 °C 4% paraformaldehyde. The brain tissues were rapidly collected and postfixed with 4% paraformaldehyde for 48 h and then with 30% sucrose for 48 h. Slices with 35-μm thicknesses were obtained from areas in the hippocampus and the prefrontal cortex. The IHC technique was performed according to the manufacturer's instructions in the kit (SA1055, Boster). The primary antibodies were anti-$A\beta_{42}$ (1:500, Cell Signaling Technology), LRP-1 (ab92544, 1:500, Abcam), RAGE (ab3611, 1:500, Abcam), and BACE1 (1:1000, Cell Signaling Technology) antibodies. The images were taken by the Leica MPS 60 instrument. Then, the images were observed at 400 × magnification. Photomicrographs were saved as TIF files, and the optical density was analyzed with the ImageJ software [31].

Statistical Analysis

Data in this study were given as the mean ± SD. Statistical significance was set at $P < 0.05$ with double-sided inspection. All analyses were performed using the SPSS 13.0 software. Graphs were drawn with GraphPad Prism version 5.01 for Windows (GraphPad Software). One-way ANOVA was performed for the MWM data among multiple groups. Student's *t*-test was performed in western blotting, ELISA, qRT-PCR and IHC analysis. The relationships between two biomarkers were analyzed by linear correlations with the correlation coefficient *r*.

Results

Spatial Memory was Impaired in the MWM Test After Chronic Sleep Restriction

In the MWM test, the escape latency continually decreased over the first 4 days of the training phase. After 21 days of CSR, performance of the MWM was significantly worse than that of the corresponding control rats on the same testing day (Fig. 1a; day 2: $p < 0.001$; day 3: $p < 0.001$; day 4: $p < 0.001$), but the escape latency to reach the platform was not significantly different between the CSR group and the control group on day 1 (Fig. 1a). Furthermore, there was no significant difference in swimming speed across the training days between the CSR rats and control rats (7th day: 30 ± 3 s vs. 29 ± 3 s, $P = 0.773$; 14th day: 29 ± 1 s vs. 28 ± 3 s, $P = 0.580$; 21st day: 28 ± 2 s vs. 28 ± 3 s, $P = 0.754$). For the probe test on day 5, the CSR-21d rats had a less number of crossings of the area where the platform had been located previously than the control rats (Fig. 1b; 4.10 ± 0.99 vs. 2.55 ± 0.93, respectively, $P = 0.002$). However, there were no significant differences between CSR rats on the 7th day or 14th day and the corresponding control rats in either of the training or examination courses. In general, these results showed that CSR impaired spatial learning and recall functions in MWM test.

Aβ Accumulated in the Brain After Chronic Sleep Restriction

The $A\beta_{42}$ levels in the hippocampus and prefrontal cortex were detected by IHC analysis and ELISA analysis. $A\beta_{42}$-positive neurons in the hippocampus and prefrontal cortex

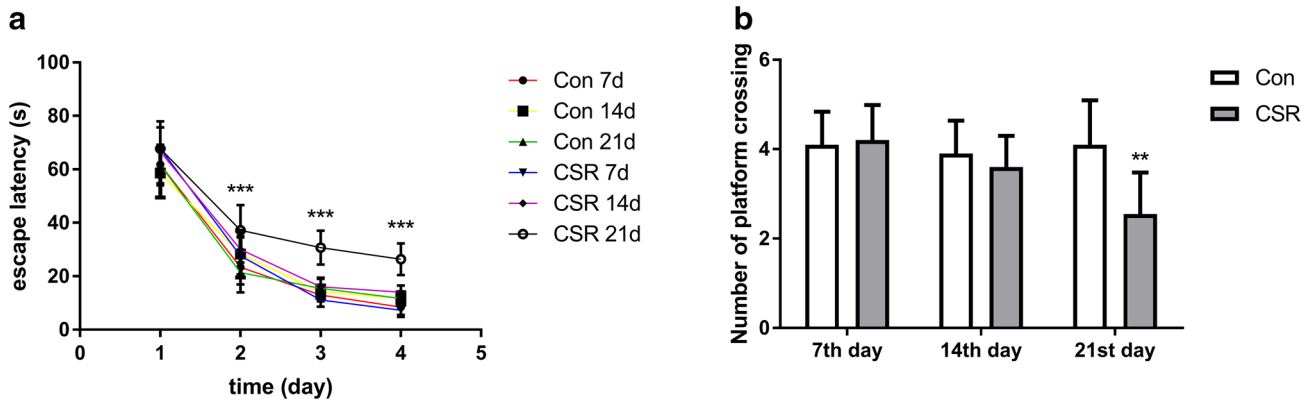

Fig. 1 Spatial learning and memory of WT rats in the MWM test was impaired after 21 days of CSR. **a** The time needed to locate the hidden platform during the spatial memory learning phase significantly increased in CSR-21d rats. **b** The number of platform crossings decreased after 21 days of CSR in the probe trial conducted on day 5. (CSR, chronic sleep restriction; Con, control). Mean ± SD, n (Control) = 12, n (CSR) = 12 (* vs. the control group, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$)

increased in the CSR rats on the 21st day compared with the corresponding control rats (Fig. 2a, b, c; hippocampus: $P=0.008$, prefrontal cortex: $P=0.001$). $A\beta_{42}$ levels significantly increased in both the hippocampus and the prefrontal cortex in CSR-21d rats compared with the control rats according to the ELISA results (Fig. 2d; hippocampus: $P=0.002$, prefrontal cortex: $P=0.011$). However, the $A\beta_{42}$ levels were not significantly different between the CSR rats of the 7th day or the 14th day and control rats according to the ELISA and IHC analysis. These results indicated that $A\beta_{42}$ deposition in the hippocampus and the prefrontal cortex was high after 21 days of sleep restriction in WT rats.

BACE1 Pathway was Upregulated After Chronic Sleep Restriction

To investigate the production of $A\beta_{42}$, we detected the levels of APP, BACE1, sAPPα and sAPPβ. Figure 3a shows the representative WB for APP protein in both the hippocampus and the prefrontal cortex. As shown in Fig. 3b, APP expression was not significantly different between the control group and the CSR group in either the hippocampus or the prefrontal cortex, and APP mRNA levels were not significantly different, as shown in Fig. 3c. This finding indicated that CSR had little effect on APP levels.

Next, we examined the levels of BACE1, sAPPα and sAPPβ. As shown in Fig. 3d–f, after 21 days of CSR, BACE1 expression significantly increased compared with the Con-21d rats, according to the IHC images (hippocampus: $P=0.021$; prefrontal cortex: $P=0.001$).

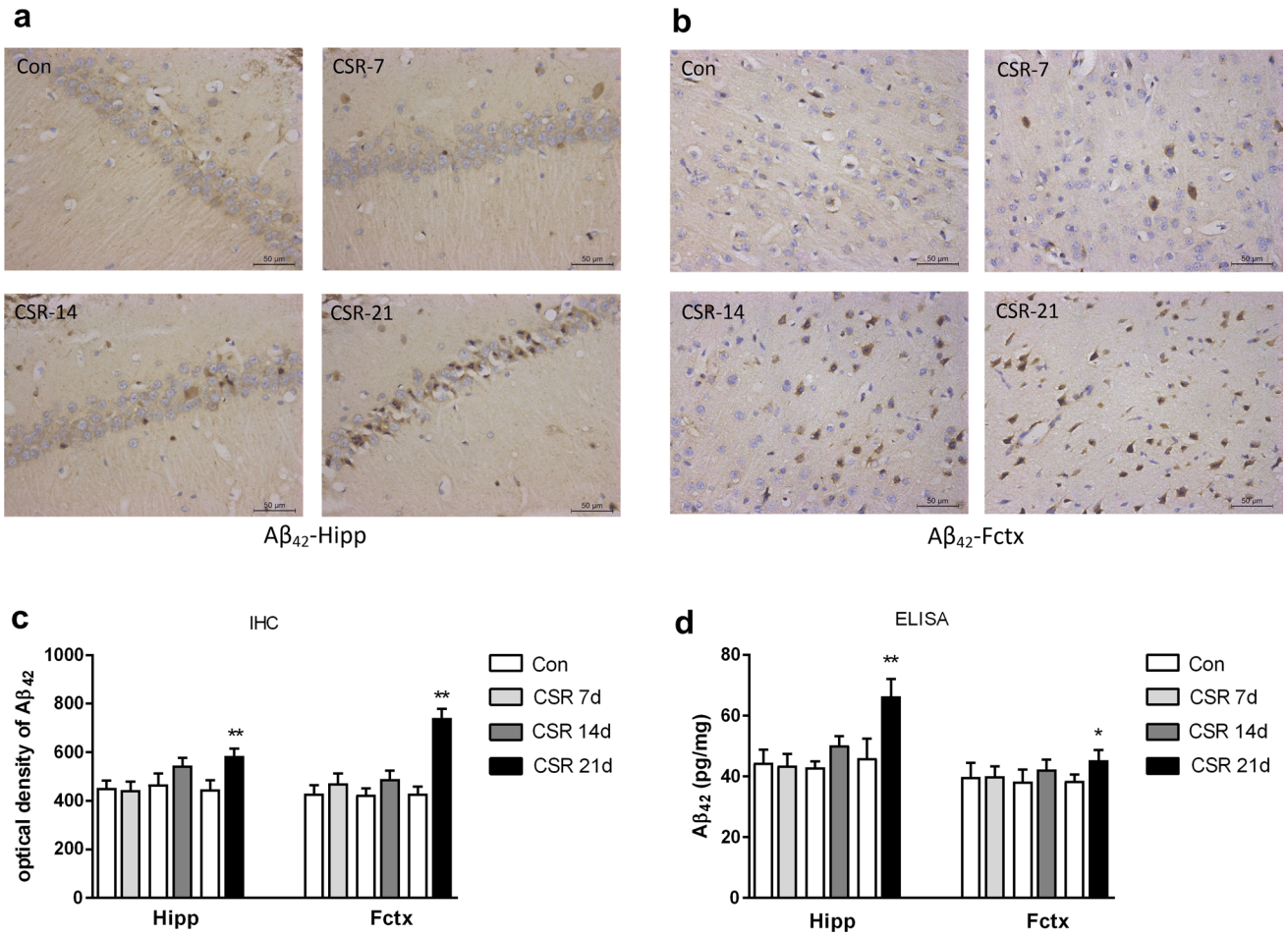

Fig. 2 $A\beta_{42}$ deposition in the hippocampus and the prefrontal cortex increased after CSR. **a, b** Immunohistochemical images of $A\beta_{42}$ in the hippocampus (**a**) and prefrontal cortex (**b**). (One representative immunohistochemical image of the control group was used because the IHC results of the three control groups were the same. Image magnification × 400). **c** Optical densities of $A\beta_{42}$-positive neurons according to the IHC. **d** $A\beta_{42}$ protein levels in the hippocampus and prefrontal cortex. $A\beta_{42}$ levels were detected by ELISA. (*CSR* chronic sleep restriction, *Con* control, *Hipp* hippocampus, *Fctx* prefrontal cortex). Mean ± SD, n (Control) = 6, n (CSR) = 6. (* vs. the control group, $*p<0.05$, $**p<0.01$, $***p<0.001$)

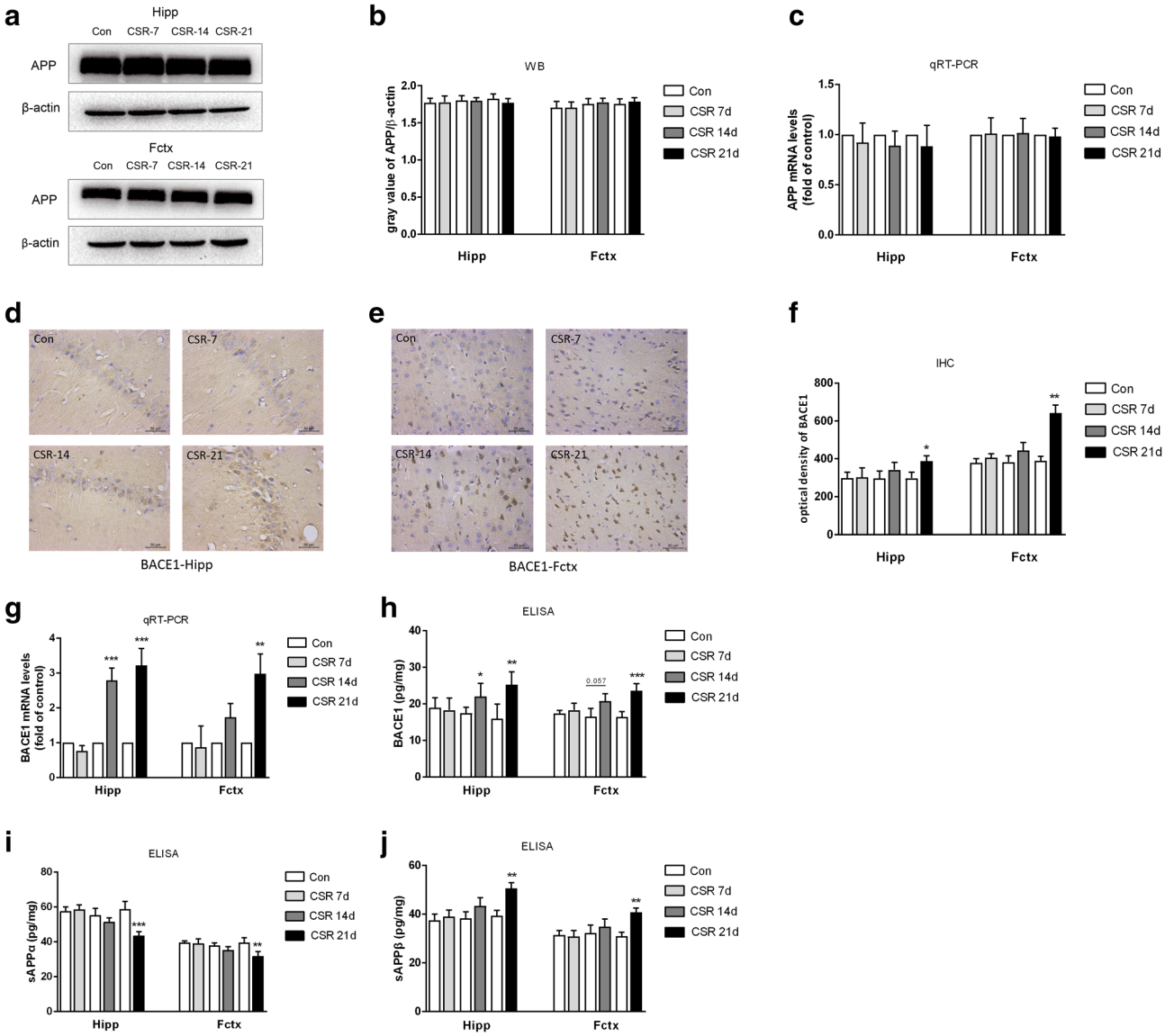

Fig. 3 Effects of CSR on the levels of proteins related to APP processing. **a, b** APP protein expression in the hippocampus and the prefrontal cortex as detected by WB (**a**) and the levels (**b**). **c** APP mRNA levels in the CSR group and control group as measured by qRT-PCR. **d, e** Immunohistochemical images of BACE1 in the hippocampus (**d**) and the prefrontal cortex (**e**). (Image magnification × 400). **f** Optical densities of BACE1-positive neurons according to IHC. **g** BACE1 mRNA levels detected by qRT-PCR. **h–j** BACE1 levels (**h**), sAPPα levels (**i**) and sAPPβ levels (**j**) in the hippocampus and the prefrontal cortex. BACE1, sAPPα, and sAPPβ levels were detected by ELISA. (*CSR* chronic sleep restriction, *Con* control). Mean ± SD, n (Control) = 6, n (CSR) = 6. (* vs the control group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

This trend was in accordance with the qRT-PCR and ELISA results (Fig. 3g; hippocampus: $P_{14d} < 0.001$, $P_{21d} < 0.001$, prefrontal cortex: $P_{21d} = 0.001$. Fig. 3h; hippocampus: $P_{14d} = 0.037$, $P_{21d} = 0.001$; prefrontal cortex: $P_{21d} < 0.001$.). Then, we examined sAPPα and sAPPβ protein expression levels. The ELISA results showed that the expression of sAPPα was lower in CSR-21d rats (Fig. 3i; hippocampus: $P < 0.001$, prefrontal cortex: $P = 0.004$) than in the control rats. However, sAPPβ expression in the CSR-21d rats significantly increased compared with that in the Con-21d rats (Fig. 3j; hippocampus: $P = 0.002$; prefrontal cortex: $P = 0.001$).

There were positive correlations between $A\beta_{42}$ and BACE1 pathway in CSR-groups (Fig. 4). According to the ELISA analysis, there was a positive correlation between the $A\beta_{42}$ level and the BACE1 level in both the hippocampus and the prefrontal cortex (Fig. 4a, b; hippocampus: $r = 0.691$, $P < 0.0001$; prefrontal cortex: $r = 0.582$, $P = 0.0002$). In contrast, the $A\beta_{42}$ level was negatively correlated with the sAPPα level (Fig. 4c, d;

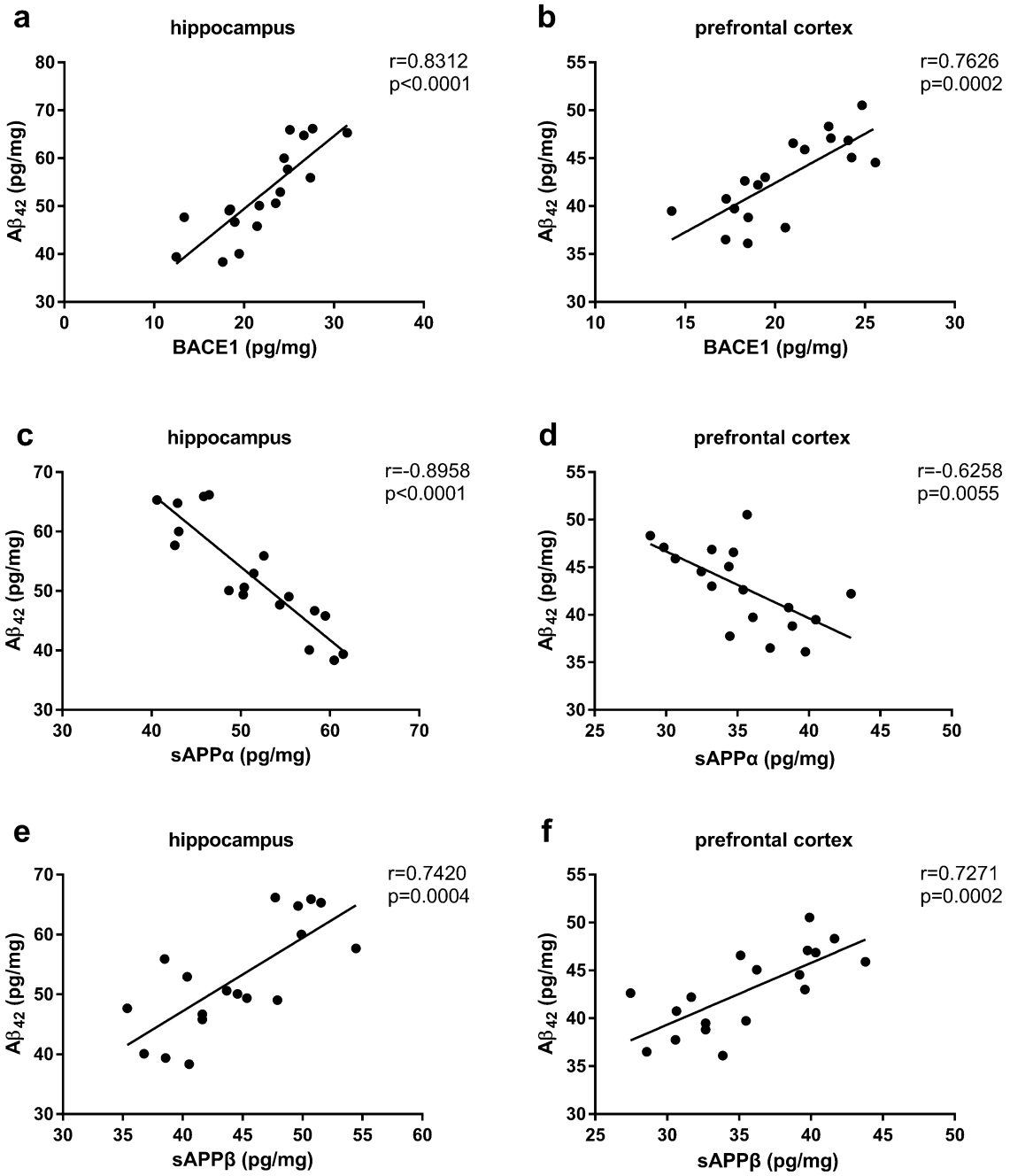

Fig. 4 The relationships between $A\beta_{42}$ and three BACE1 pathway related proteins in the hippocampus and the prefrontal cortex according to linear correlational analyses. **a, b** The positive relationships between $A\beta_{42}$ levels and BACE1 levels in the hippocampus (**a**) and the prefrontal cortex (**b**). **c, d** The negative relationships between $A\beta_{42}$ levels and sAPPα levels in the hippocampus (**c**) and the prefrontal cortex (**d**). **e, f** The positive relationships between $A\beta_{42}$ levels and sAPPβ levels in the hippocampus (**e**) and the prefrontal cortex (**f**). $A\beta_{42}$, BACE1, sAPPα and sAPPβ levels were detected by ELISA. For the linear correlational analyses, the *r* value was used to express each relationship

hippocampus: $r = 0.803$, $P < 0.0001$; prefrontal cortex: $r = 0.392$, $P = 0.0055$), whereas with continual increases in sAPPβ levels, the levels of $A\beta_{42}$ also increased (Fig. 4e, f; hippocampus: $r = 0.551$, $P = 0.0004$; prefrontal cortex: $r = 0.529$, $P = 0.0002$). From this correlative analysis we

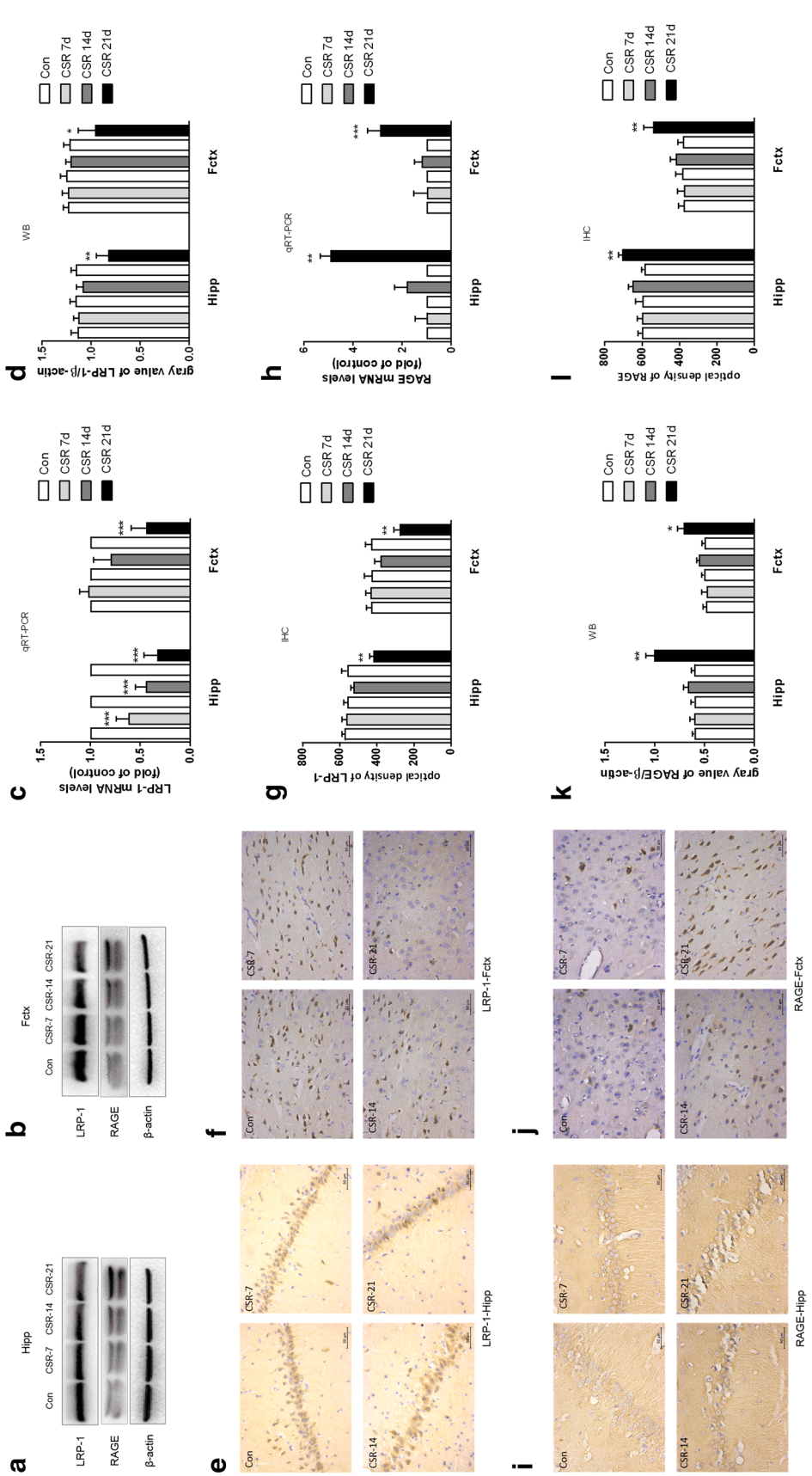

Fig. 5 The levels of LRP-1 and RAGE expression. **a, b** WB bands of LRP-1 and RAGE proteins in the hippocampus (**a**) and the prefrontal cortex (**b**). **c** The LRP-1 mRNA levels in the hippocampus and the prefrontal cortex significantly decreased after CSR, as determined by qRT-PCR. **d** LRP-1 levels quantitated by WB. **e, f** Immunohistochemical images of LRP-1 in the hippocampus (**e**) and the prefrontal cortex (**f**). **g** Optical densities of LRP-1-positive neurons, according to the IHC statistical analyses. **h** The RAGE mRNA concentrations measured by qRT-PCR significantly increased after CSR. **i, j** Immunohistochemical images of RAGE in the hippocampus (**i**) and the prefrontal cortex (**j**). **k** RAGE levels quantitated by WB. **l** Optical densities of RAGE-positive neurons measured by IHC statistical analyses. (Image magnification × 400). (*CSR* chronic sleep restriction, *Con* control). Mean ± SD, n (Control) = 6, n (CSR) = 6. (* vs the control group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

could conclude that the activated BACE1 pathway may have effects on $A\beta_{42}$ increase in CSR rats.

Brain Aβ Transport was Disrupted After Chronic Sleep Restriction

To assess Aβ transportation, we analyzed the levels of LRP-1 and RAGE in the brain. Figure 5a, b shows the representative WB of every target protein. The mRNA levels of LRP-1 decreased in the hippocampus and the prefrontal cortex of CSR rats compared to those of the control rats (Fig. 5c; hippocampus: $P_{7d} < 0.001$, $P_{14d} < 0.001$, $P_{21d} < 0.001$; prefrontal cortex: $P_{21d} < 0.001$). As shown in Fig. 5d, the LRP-1 concentration was significantly reduced in the hippocampus and the prefrontal cortex of CSR rats on the 21st day according to the WB analysis (hippocampus: $P = 0.001$; prefrontal cortex: $P = 0.012$). Figure 5e, f shows the IHC images of LRP-1-positive cells in the hippocampus and the prefrontal cortex, and the corresponding statistical analysis of the optical density of positive cells showed a consistent tendency (Fig. 5g; hippocampus: $P = 0.002$; prefrontal cortex: $P = 0.004$).

In contrast to the LRP-1 levels, the mRNA levels of RAGE increased in the hippocampus and the prefrontal cortex of CSR-21d rats compared to those of the control rats (Fig. 5h; hippocampus: $P = 0.004$; prefrontal cortex: $P < 0.001$). According to the WB, the RAGE protein levels increased significantly in the hippocampus and the prefrontal cortex of CSR-21d rats compared to those of the control rats (Fig. 5a, b, k; hippocampus: $P = 0.008$; prefrontal cortex: $P = 0.014$). The IHC images and the corresponding statistical analyses of RAGE-positive cells in the hippocampus and the prefrontal cortex showed a consistent tendency (Fig. 5i, j, l; hippocampus: $P = 0.001$; prefrontal cortex: $P = 0.008$).

To explore the relationship between transporters and $A\beta_{42}$, we performed correlational analyses. According to WB, the LRP-1 levels and the $A\beta_{42}$ levels were negatively correlated in both the hippocampus and the prefrontal cortex (Fig. 6a, b; hippocampus: $r = 0.544$, $P = 0.0005$; prefrontal cortex: $r = 0.379$, $P = 0.0066$). In contrast, the RAGE levels and the $A\beta_{42}$ levels had a positive relationship (Fig. 6c,

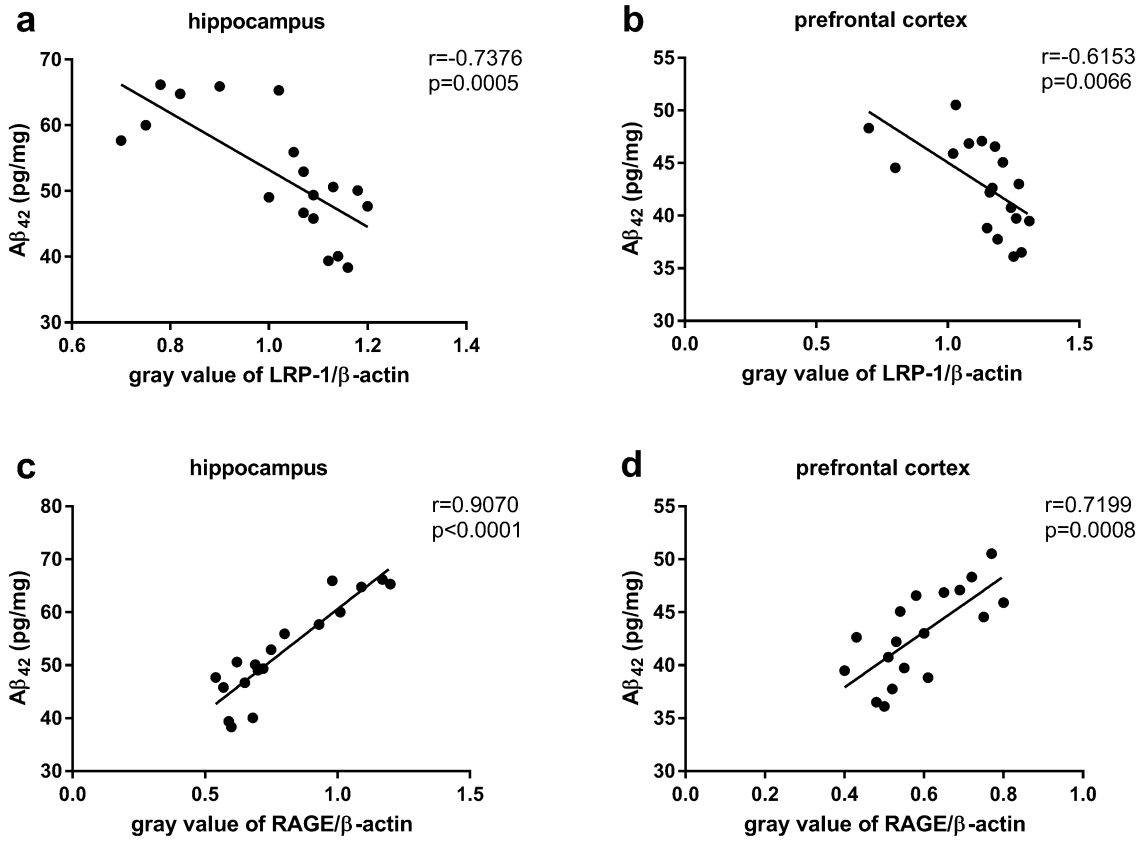

Fig. 6 The relationships between $A\beta_{42}$ and transporters in the hippocampus and the prefrontal cortex. **a, b** The negative relationships between $A\beta_{42}$ levels and LRP-1 levels in the hippocampus (**a**) and the prefrontal cortex (**b**). **c, d** The positive relationships between $A\beta_{42}$ levels and RAGE levels in the hippocampus (**c**) and the prefrontal cortex (d). $A\beta_{42}$ levels were detected by ELISA, and LRP1 levels and RAGE levels were detected by WB. For the linear correlational analyses, the *r* value was used to express each relationship

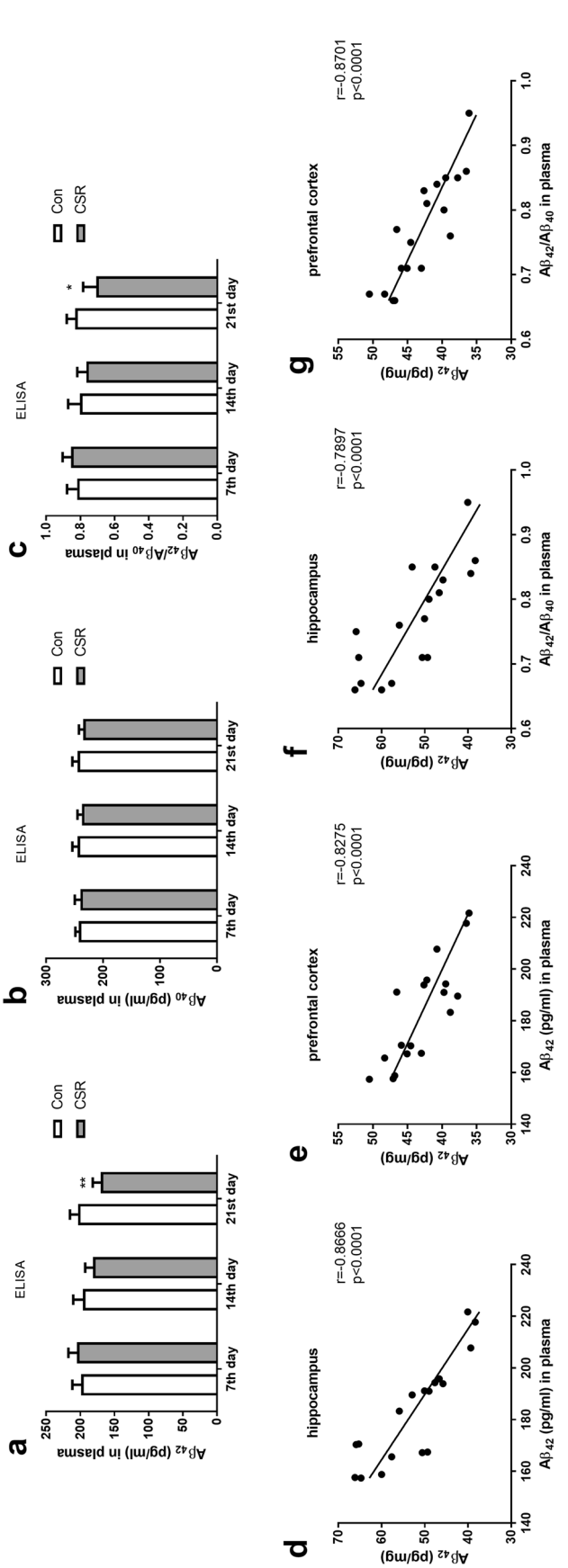

Fig. 7 The relationships between plasma Aβ levels and brain $A\beta_{42}$. **a, b** Plasma $A\beta_{42}$ levels (**a**) and $A\beta_{40}$ levels (**b**) in CSR rats and control rats. **c** The ratios of plasma $A\beta_{42}/A\beta_{40}$ in CSR rats and control rats. Plasma $A\beta_{42}$ and $A\beta_{40}$ levels were detected by ELISA. **a**–**c** Mean ± SD, n (Control) = 6, n (CSR) = 6. (* vs. the control group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). **d** The negative relationship between plasma $A\beta_{42}$ levels and hippocampal $A\beta_{42}$ levels. **e** The negative relationship between plasma $A\beta_{42}$ level and prefrontal cortex $A\beta_{42}$ levels. **f** The negative relationship between the $A\beta_{42}/A\beta_{40}$ ratio and hippocampal $A\beta_{42}$ levels. **g** The negative relationship between the $A\beta_{42}/A\beta_{40}$ ratio and prefrontal cortex $A\beta_{42}$ levels. **d**–**g** For the linear correlational analyses, the *r* value was used to express each relationship. (*CSR* chronic sleep restrictionm *Con* control)

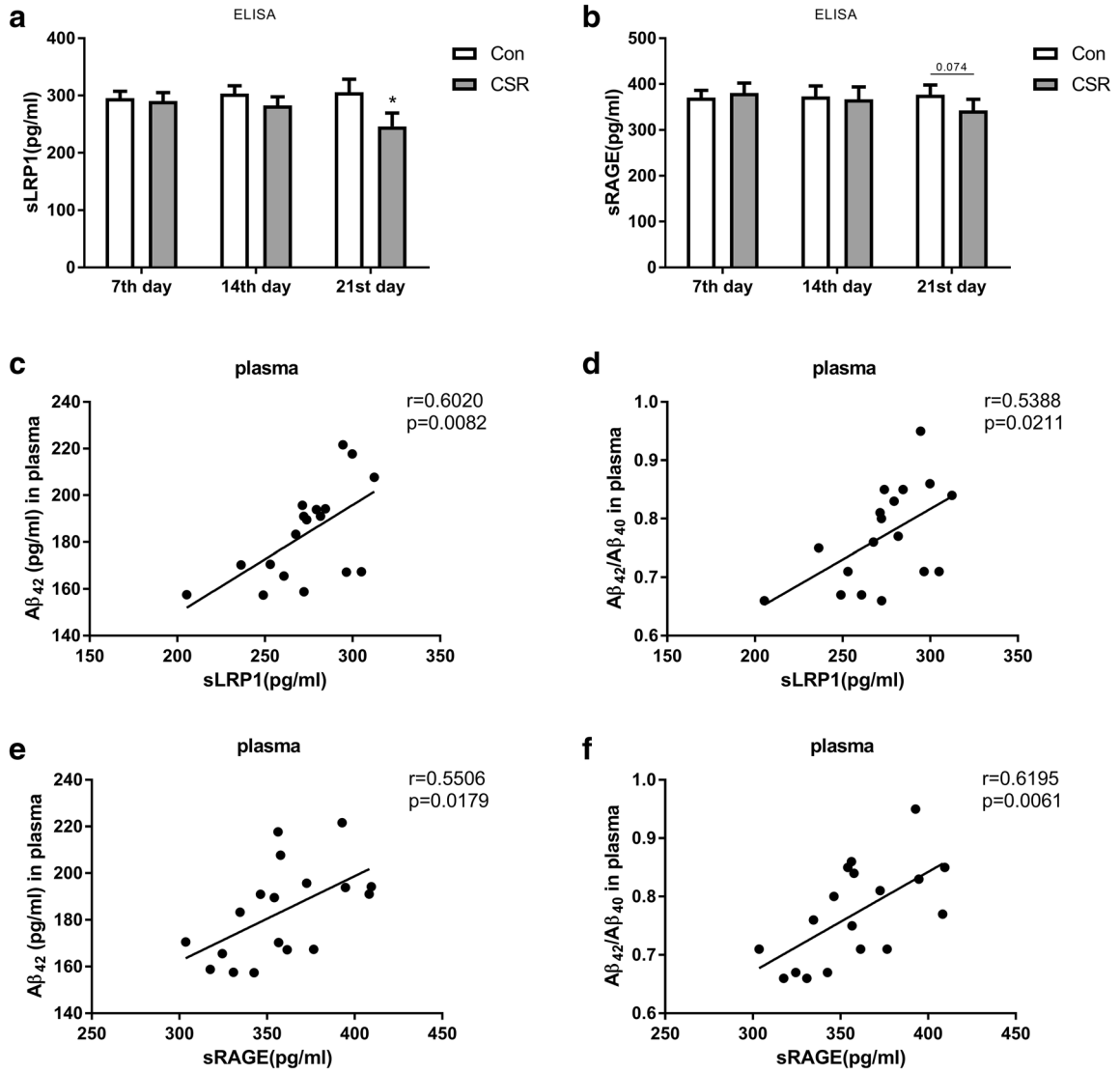

Fig. 8 The relationships between plasma $A\beta_{42}$ levels and Aβ transporters in plasma. **a, b** Plasma sLRP1 levels (**a**) and sRAGE levels (**b**) in CSR rats and control rats. Plasma sLRP1 and sRAGE levels were detected by ELISA. Mean ± SD, n (Control) = 6, n (CSR) = 6. (* vs. the control group, $*p<0.05$, $**p<0.01$, $***p<0.001$). **c, d** The positive relationships between plasma $A\beta_{42}$ levels (**c**), the plasma $A\beta_{42}/A\beta_{40}$ ratio (**d**) and plasma sLRP1 levels. **e, f** The positive relationships between plasma $A\beta_{42}$ levels (**e**), the plasma $A\beta_{42}/A\beta_{40}$ ratio (**f**) and plasma sRAGE levels. For the linear correlational analyses, the *r* value was used to express each relationship. (*CSR* chronic sleep restriction, *Con* control)

d; hippocampus: $r=0.823$, $P<0.0001$; prefrontal cortex: $r=0.518$, $P=0.0008$). Significant correlations between $A\beta_{42}$ and transporters demonstrated that the disrupted clearance of $A\beta_{42}$ in brain had contribution to the CSR-induced $A\beta_{42}$ accumulation.

Plasma $A\beta_{42}$ Transport was Disturbed After Chronic Sleep Restriction

Figure 7 shows the plasma Aβ values of the CSR and control rats, and the correlation between plasma Aβ levels and $A\beta_{42}$ levels in the hippocampus and the prefrontal cortex. The plasma $A\beta_{42}$ concentration decreased significantly only in the CSR-21d rats compared to the control rats (Fig. 7a, p = 0.004). Plasma $A\beta_{40}$ levels were not significantly different between the CSR rats and control rats (Fig. 7b), while the ratio of $A\beta_{42}/A\beta_{40}$ decreased significantly in the CSR-21d rats compared to that in the controls (Fig. 7c, p = 0.012).

To explore if there was a relationship between plasma Aβ levels and brain $A\beta_{42}$ in the CSR groups, we performed correlational analyses. The results showed that, according to the ELISA analysis, there was a negative correlation between plasma $A\beta_{42}$ levels and brain $A\beta_{42}$ levels (Fig. 7d, e; hippocampus: $r=0.751$, $P<0.0001$; prefrontal cortex:

$r = 0.685$, $P < 0.0001$). Similarly, the ratio of $A\beta_{42}/A\beta_{40}$ was negatively correlated with brain $A\beta_{42}$ levels in the hippocampus (Fig. 7f: $r = 0.624$, $P < 0.0001$) and prefrontal cortex (Fig. 7g: $r = 0.757$, $P < 0.0001$). However, there was no correlation between plasma $A\beta_{40}$ and brain $A\beta_{42}$ levels.

To investigate whether the transport disturbances in the periphery were involved in the changes in plasma Aβ levels, we measured circulating sLRP1 and sRAGE. The results showed that the plasma concentrations of sLRP1 decreased significantly after 21 days of CSR compared to those in control rats, according to the ELISA (Fig. 8a, $P = 0.019$). For sRAGE (Fig. 8b), even though a slight decrease after CSR was observed, no significant effect of CSR was found. Additionally, the concentrations of plasma $A\beta_{42}$ and the ratio of plasma $A\beta_{42}/A\beta_{40}$ were positively correlated with the plasma levels of sLRP1 and sRAGE in CSR rats (Fig. 8c–f).

Discussion

It has been reported that poor sleep quality is one of the risk factors for AD, but the mechanism underlying the contribution of sleep disturbances to AD has not been elucidated. In the present study, we found that 21 days of CSR induced cognitive dysfunction in rats during the MWM test, and $A\beta_{42}$ deposition in the hippocampus and the prefrontal cortex was elevated. Meanwhile, CSR increased the expression of BACE1 and sAPPβ and decreased the sAPPα levels in both the hippocampus and the prefrontal cortex. Moreover, 21 days of CSR induced LRP-1 level decrease and RAGE increase in both the hippocampus and the prefrontal cortex. In addition, 21 days of CSR decreased plasma $A\beta_{42}$ levels and sLRP1, and plasma $A\beta_{42}$ concentrations were linearly correlated with plasma sLRP1 and sRAGE levels.

As far as we know, senile plaques are the key characteristic of AD, and the main component of these plaques is Aβ. A previous study in fruit flies showed that sleep deprivation could result in Aβ deposition in the brain [32], and the Aβ levels were higher after acute or chronic sleep restriction in rodents [26, 27]. Spira et al. also found that cognitively normal elderly individuals who had poor quality of sleep were more prone to have cerebral Aβ plaque pathology [33]. These studies suggested that sleep disorders might indicate future cerebral Aβ deposition or even development of dementia. Consistent with these previous studies, the present study demonstrated that CSR would induce cognitive dysfunction and increase Aβ deposition in both the hippocampus and the prefrontal cortex.

There is a large amount of evidence indicating that the mass accumulation of toxic Aβ is caused by a production and clearance imbalance [4]. There are two pathways, namely, the amyloidogenic and nonamyloidogenic pathways, for APP processing [34]. One of the products of the amyloidogenic pathway is sAPPβ, while sAPPα is one of the products of the nonamyloidogenic pathway. In the process of $A\beta_{42}$ production, BACE1 is the most important β-secretase that regulates $A\beta_{42}$ production [35]. BACE1 is generally described as a rate-limiting enzyme and a strong promoter of $A\beta_{42}$ production. In the present study, 21 days, and even 14 days, of CSR could increase the levels of BACE1 protein and sAPPβ protein and decrease sAPPα levels in both the hippocampus and the prefrontal cortex. Additionally, $A\beta_{42}$ deposition was positively correlated with BACE1 and sAPPβ levels and negatively correlated with sAPPα levels. These findings indicated that CSR may increase Aβ production by decreasing the nonamyloidogenic pathway and increasing the amyloidogenic β-secretase pathway. A previous study found that APP exerted neurotrophic active effects by promoting cell proliferation, differentiation and neurite growth and could regulate stem cell proliferation and differentiation. Additionally, APP could motivate synaptic plasticity, learning and memory [36]. Therefore, the results showed that CSR had no significant effect on APP levels, perhaps because APP had several physiological functions within cognitive function. This evidence indicated that APP might act as a kind of normal transmembrane protein and was almost unaffected by CSR. Altogether, the results suggested that it was the upregulation of the BACE1 pathway, rather than APP levels, that contributed to Aβ deposition in the CSR rats.

There are several mechanisms of cerebral Aβ clearance, including microglial phagocytosis, interstitial fluid drainage, and transport of Aβ across the BBB into the peripheral blood, which is mainly driven by LRP-1 and RAGE in vascular endothelial cells. LRP-1 is highly expressed in many kinds of cerebral cells, including vascular cells, neurons, and neural glia cells. LRP-1 is a member of the low-density lipoprotein receptor (LDLR) family and can bind to various kinds of ligands, including α2-macroglobulin, apolipoprotein E, and Aβ. Therefore, the main functions of LRP-1 are to maintain homeostasis of the brain and to control the dynamic Aβ equilibrium [37]. Except for mediating Aβ clearance from the brain to the cerebral plasma, the LRP-1 cytoplasmic C-terminal domain could interrelate and interact with APP's cytoplasmic domain, which in turn affects APP processing and Aβ creation [38].

RAGE plays an important role in preventing Aβ clearance by regulating the influx of peripheral Aβ into the brain [39]. Moreover, Aβ–RAGE interactions could damage tight junctions of the BBB and induce destruction of BBB structural integrity, and the resulting faulty BBB could lead to leakage of multiple toxic substances, including Aβ, into the brain [40]. Additionally, a previous study found that reductions in RAGE activity could inhibit Aβ from aggregating in the cerebrum and prevent Aβ neurotoxicity [12]. In the present study, we found that 21 days of CSR induced LRP-1 level reductions and RAGE

elevations in both the hippocampus and the prefrontal cortex, and $A\beta_{42}$ deposition was positively correlated with RAGE levels and negatively correlated with LRP-1 levels. These results indicated that Aβ transport disturbance was involved in CSR-induced Aβ accumulation.

Since inflammation played an important role in the AD process [41], and neuroinflammation mediators, such as IL-1β and TNF-α, could increase BACE1 levels and stimulate amyloidogenic APP processing to facilitate Aβ accumulation in the brain [42, 43], and could affect LRP-1 and RAGE and thus lead to Aβ clearance disruption [44]. Moreover, previous studies reported that pro-inflammatory cytokines levels in brain and plasma increased after sleep deprivation in both humans and animal model [45, 46]. So in order to determine if the inflammation or other pathways were involved in the underlying mechanisms of CSR-induced disrupted balance of Aβ production and clearance, further studies are needed.

Plasma transportation is also very important in Aβ clearance. It has been reported that the dynamic changes in Aβ between the brain and peripheral blood proved that circulating Aβ played a crucial role in AD pathology [47]. Moreover, a recent publication found that, after sleep deprivation, the $A\beta_{42}/A\beta_{40}$ ratio declined by 5.5% [48]. In the present study, we found that CSR could decrease plasma $A\beta_{42}$ levels and the concentration of plasma $A\beta_{42}$ was negatively correlated with brain $A\beta_{42}$ levels. We could deduce that the influx of $A\beta_{42}$ might lead to the decreased levels of $A\beta_{42}$ in plasma and make a contribution to the Aβ accumulation in hippocampus and prefrontal cortex. Meanwhile, plasma sLRP1 level increased and sLRP1 and sRAGE were positively correlated with plasma $A\beta_{42}$ after CSR. We concluded that the disordered expression of these two important transporters of $A\beta_{42}$ in plasma could contribute to the disturbance of plasma $A\beta_{42}$ transport. In one way, the decreased sLRP1 and sRAGE led to less bonding with $A\beta_{42}$ particles, and so more $A\beta_{42}$ will flow into brain through BBB with increased RAGE in BBB. On the other hand, decreased plasma $A\beta_{42}$ might have a feedback on transporters and contribute to the decreased level of sLRP1 and sRAGE. Taken together, these findings suggested that CSR might also induce peripheral Aβ transport disturbances, which contributed to brain Aβ deposition. However, plasma $A\beta_{42}$ levels decrease after CSR may also result from deposition of $A\beta_{42}$ in the brain.

In summary, our study demonstrated that CSR can lead to brain Aβ accumulation in WT rats. The underlying mechanisms of this phenomenon may be related to increased production of Aβ via upregulation of the BACE1 pathway and disrupted Aβ clearance affecting brain and peripheral Aβ transport (Fig. 9). These results indicated that CSR increased AD risk by promoting Aβ accumulation. However, the mechanism of CSR-disrupted balance of Aβ production and clearance, such as inflammation and oxidative stress, has not been determined. This mechanism needs to be studied in the future.

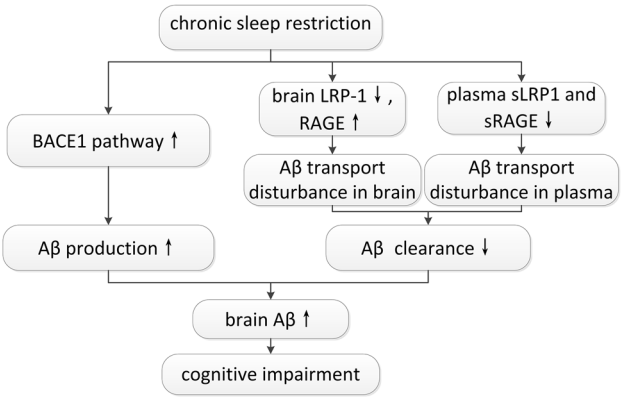

Fig. 9 The speculative mechanism of brain Aβ accumulation caused by chronic sleep restriction. ↑, increase; ↓, decrease

There are some limitations of this study. Firstly, we didn't measure plasma stress levels while stress and sleep disruption often go together and may interact and intensify Aβ aggregation. However, one previous study, in which the sleep protocol is the same as ours, has demonstrated that plasma corticosterone levels increased after 1 and 6 days of sleep restriction and recovered to the baseline after 4 h of sleep recovery, and it seemed to prove that the stress caused by this sleep restriction protocol was transient and can't have effects on cognitive function [28]. Moreover, eliminating the adrenal stress response could not affect sleep deprivation-induced learning and memory disability in the water maze [49]. To make it clear, the effects of stress on cognitive impairment and Aβ aggregation should be investigated in the future. Secondly, the present study is observational and only found that BACE1, LRP-1 and RAGE, were associated with the Aβ accumulation induced by CSR, but it may not be causal of Aβ accumulation in the brain. The changes of BACE1, LRP-1 and RAGE may also secondary to Aβ accumulation or other pathways. Further studies related to interventional experiments are needed. Thirdly, since the advancing age is a key risk factor in AD as we all know, so in order to avoid the effect of old age on cognition, we used 10-month old adult rats to concentrate on the effects of CSR on cognitive function. This may be one of reasons why it took a long time to perform cognitive impairment in CSR-rats. Another reason might be that AD is a slowly progressing disorder and it must be a long process to eventually develop to cognitive impairment with any potential risk factors added. Moreover, we will do studies in the future to illustrate the effect of age-related CSR on cognition.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to disclose.

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