

Epigenetic modulation of Cdk5 contributes to memory deficiency induced by amyloid fibrils

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Abstract Alzheimer's disease (AD) is a frequent neurodegenerative disorder with progressive neuroinflammation, loss of synaptic plasticity in central neurons and memory deficiency. Numerous studies demonstrated the epigenetic modification of the expression of specific genes involved in the pathogenesis of amyloid-associated memory deficiency. It was also reported that dysregulation of cyclin-dependent kinase 5 (Cdk5) activity critically contributed to the synaptic dysfunction and memory deficiency in the rodent model of AD. The present study aims to study the epigenetic mechanism underlying the altered Cdk5 activity and its functional significance in the rats with hippocampal infusion of amyloid fibrils. Significantly increased mRNA and expression of Cdk5 were observed in the hippocampal CA1 in the rats injected with amyloid fibrils. Increased acetylation of histone H3 was detected in the *Cdk5* promoter region in hippocampal CA1 in these rats. Further chromatin immunoprecipitation and bisulfite sequencing studies illustrated the decreased cytosine methylation in the *Cdk5* promoter region in hippocampal CA1 in the modeled rats. Administration with Cdk5 inhibitor roscovitine significantly attenuated the phosphorylation of tau, recovered the synaptic dysfunction of hippocampal CA1

neurons, and improved the behavioral performance in the Morris water maze test and novel object recognition test in the rats injected with amyloid fibrils. These results elucidate the potential epigenetic mechanism underlying the upregulated expression of Cdk5 induced by amyloid fibrils and provided novel insights into the pathogenic mechanism of Alzheimer's disease.

Keywords Amyloid fibrils · Cyclin-dependent kinase 5 · Epigenetic · Tau · Synaptic plasticity · Memory deficiency

Introduction

Alzheimer's disease is a frequent neurodegenerative disorder with progressive neuroinflammation, neuronal death, loss of synaptic plasticity in central neurons and memory deficiency. Recently, a line of evidences have pointed out that epigenetic modification of the specific loci in the genome, particularly the altered histone acetylation and DNA methylation, may substantially alter the hippocampal synaptic plasticity and memory function, thus contributing to the loss of neurocircuits and memory deficiency in the patients and rodent models of AD (van den Hove et al. 2014). Acetylation of N-terminus lysine residue, which is catalyzed by histone acetyltransferases, changes the overall charge of the histone tail from positive to neutral, which increasing the accessibility of transcription factors to the DNA and facilitating the transcription of target gene (Adwan and Zawia 2013). Previous studies demonstrated that overexpression of histone deacetylase 2, which decreased histone acetylation and generally suppressed transcription of target genes, substantially contributed to the hippocampal synaptic dysfunction and memory deficiency in the rodent models (Graff et al. 2012; Guan et al.

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2009). Methylation on the 5'-cytosine in CpG rich regions, catalyzed by DNA methyltransferases in the presence of the methyl donor S-adenosylmethionine (Adwan and Zawia 2013), is generally associated with gene suppression by impeding the transcriptional factor and recruiting transcriptional repressor such as methyl CpG binding protein 2 (Graff et al. 2011). Alteration of genomic methylation remarkably impaired the synaptic plasticity in central neurons and cognitive performance in the mice (Feng et al. 2010) and was observed in several brain regions in the AD patients (Mastroeni et al. 2009). The present study aims to further elucidate the epigenetic mechanism in the synaptic dysfunction and memory deficiency in the rat model of AD.

While previous evidences showed that serine/threonine kinase cyclin-dependent kinase 5 (Cdk5) is crucial for neuronal migration, neuronal differentiation, synapse development and synaptic function in the physiological conditions, aberrant activation of Cdk5 plays a pivotal role in has long been associated with the pathophysiology of numerous neurodegenerative diseases (Cheung and Ip 2012). The activation of Cdk5 depends upon its association with the activator p35 or p39, or the truncated p25 or p29, which has a restricted expression in the nervous system (Cruz and Tsai 2004). It was reported that increased Cdk5-p25 activity significantly contributed to the neuronal loss and memory deficiency induced by amyloid fibrils (Cruz and Tsai 2004). Activation of Cdk5 by amyloid fibrils demonstrated the potency to mediate local phosphorylation of tau, which resulting in dissociation of tau from microtubules and the formation of intracellular neurofibrillary tangles (Zempel et al. 2010). Hence, the present study aims to elucidate the epigenetic mechanism underlying upregulation of hippocampal Cdk5, and its involvement in synaptic dysfunction and memory deficiency in the rat model of AD.

Materials and methods

Animals

Adult male Wistar rats (200–220 g, about 10 weeks) were obtained from the Institutional Center of Experiment Animals and were housed in the standard lab conditions (22 ± 2 °C and 12:12 h light cycle) with access to food and water ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee, and were carried out following the guidelines of National Institution of Health.

Microinjection of amyloid fibrils and other reagents

The rats were anesthetized with sodium pentobarbital (45 mg/kg, ip), and a 30 gauge stainless steel cannula

with a 33 gauge stylet plug was bilaterally implanted 0.5 mm above the dorsal hippocampus (-3.5 mm AP, ± 2.0 mm ML and -2.7 mm DV, according to Bregma) (Ahmed et al. 2010; Chacon et al. 2004). The rats were allowed 1 week recovery prior to further study. Amyloid ($A\beta_{1-42}$) fibrils (20 μ g in 1 μ l per side) were formed as described previously and delivered into the hippocampal CA1 area with a 33 gauge injector at a rate of 0.5 μ l/min. Same dose of reversed peptide ($A\beta_{42-1}$) was applied in the rats in control group (Chacon et al. 2004). Morris water maze test and novel object recognition test were performed 7 days after the injection of amyloid fibrils to detect the memory function in the rats. In some groups of rats, roscovitine (5 μ g in 1 μ l) was delivered into the hippocampal CA1 for 3 days prior to the behavioral tests, and the last injection of roscovitine was performed 1 day prior to the behavioral tests.

Morris water maze test

Spatial learning and memory were assessed with Morris water maze test in the rats of all appropriate groups (Typlt et al. 2013). Briefly, a circular pool (1.8 m in diameter) was filled with opaque water (45 cm deep) at room temperature (24 °C), and a platform (15 cm in diameter) was submerged 2 cm below the water surface in the center of the target quadrant. Surrounding permanent visual cues existed for spatial orientation. The animal received training sessions for five consecutive training days, and each training session included four trails with different starting position and 15-minute inter-trail intervals. The animals were guided to the platform if they did not locate it within 120 s. The animals then stayed on the platform for 20 s and returned to the home cages. The time for animal spent to reach the platform was recorded as the escape latency in each trial. On the seventh day, the platform was removed and the rats were allowed to swim in the maze for 60 s for a probe trail. The activity of animals was recorded and tracked using the Biobserve Viewer software (Germany).

Novel object recognition test

Novel object recognition test, consisting of three phases of habituation, acquisition trial, and testing trial, was performed in the rats with the previously reported protocol (Smith et al. 2013). Rats were habituated in an open-field arena for 20 min on three consecutive days under dim ambient light conditions. The activity of rats was recorded with a video camera. In an acquisition trial, two identical objects were placed in diagonally opposite corners of the chamber 8–9 cm from the walls. A rat was placed at the midpoint between the objects. After allowing 10 min to explore the objects, the rat was returned to the home cage.

The testing trail was performed 4 h after the acquisition trail. In the testing trail, one of the objects was replaced with a novel object of the same height and volume but different shape and appearance. For testing, the rat was again placed in the chamber to explore the objects for 3 min. The amount of time spent exploring each object (nose sniffing and head orientation within <1.0 cm) was recorded and evaluated by operators blinded to the treatment. All objects and the test box were cleaned with 70 % ethanol between rats to eliminate the odor cues. Novel preference was calculated by the time exploring on the novel object divided by total exploration time on both novel and familiar objects and was compared among the groups.

Chromatin immunoprecipitation (ChIP) Assay

The ChIP assay was performed as previously described with minor modifications (Lian et al. 2013). The hippocampal CA1 tissues were collected from the rats immediately after the behavioral tests and fixed in 1 % formaldehyde for 5 min at room temperature. Chromatin was extracted and sonicated on ice 6×10 s to obtain fragments of approximately 300–400 bp. A tenth of the lysates was saved as input DNA. The polyclonal antibody against acetylated histone H3 antibody (1:100, Millipore) was added to each sample and incubated overnight at 4 °C with gentle mixing. Rabbit IgG and monoclonal anti-RNA polymerase II antibody (1:100, Millipore) was used as the negative and positive control. Immunocomplexes were collected by salmon sperm DNA/protein Agarose beads. DNA/protein complexes were eluted by adding 200 μ l fresh elution buffer (0.1 M NaHCO₃/1 % SDS) with 10 min rotation at RT. After reversion of cross-links, the DNA fragments were purified with phenol–chloroform extraction followed by acid ethanol precipitation.

Real-time PCR was performed to amplify fragments (about 200 bp) within the transcriptional control region of target genes. Primer sets were used as following: *Cdk5* (5'-TGCTTCCTGGGAGTTGAAGT-3', 5'-CGGCCATGTTTCCCAAGATT-3'), *Gapdh* (5'-AGACAGCCGCATCTTCTTGT-3', 5'-CTGCGGGAGAAGAAAGTCAG-3'). The ChIP signal was analyzed as the following method in the handbook provided by the manufacturer: $\Delta C_{t[\text{normalized ChIP}]} = (C_{t[\text{ChIP}]} - (C_{t[\text{Input}]} - \text{Log}_2(\text{input dilution factor})))$; and ChIP/Input ratio (ChIP %) was calculated as $2^{(-\Delta C_{t[\text{normalized ChIP}]})}$.

Methylated DNA immunoprecipitation (MeDIP) assay

The MeDIP assay was performed as previously described with minor modification (Provencal et al. 2013). Briefly, hippocampal CA1 tissue was homogenized in the lysis buffer and genomic DNA was sonicated on ice 8×10 s. Sonicated samples were centrifuged at 14,000g for 10 min,

and the supernatants were collected. The polyclonal antibody against 5-methylcytosine (1:100, Millipore) was added to each sample and incubated overnight at 4 °C with gentle mixing. The DNA-antibody complex was enriched with protein A agarose beads. DNA fragments in the input and pulled-down fractions were then purified with phenol–chloroform extraction followed by acid ethanol precipitation. Real-time PCR was performed to amplify about 200-bp segments corresponding to CpG sites within *Crh* promoter region. Primer sets were used as following: *Cdk5* (5'-TGCTTCCTGGGAGTTGAAGT-3', 5'-CGGCCATGTTTCCCAAGATT-3'), *Gapdh* (5'-AGACAGCCGCATCTTCTTGT-3', 5'-CTGCGGGAGAAGAAAGTCAG-3'). Amplifications were run in triplicate, and the PCR data were analyzed as above.

Quantitative real-time (RT) PCR

Hippocampal CA1 tissue was sampled, and the total RNA was extracted with TRIzol reagent (Invitrogen). Total cDNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen), and real-time PCR was performed in triplicate. The sequences of the primers to measure mRNA of *Cdk5*: 5'-TTGGGGCAGACGAGAAAGCGC-3' and 5'-TCACGTGCTCAAAAGTGTCAG-3'. The primers for GAPDH mRNA were as follows: 5'-AGACAGCCGCATCTTCTTGT-3' and 5'-CTTGCCGTGGGTAGAGTCAT-3'. Fold change was calculated using the $\Delta\Delta C_{t}$ method and compared between the appropriate groups.

Bisulfite sequencing PCR

DNA samples were prepared from the substantia nigra, purified, processed for the bisulfite modification with the EZ DNA Methylation-Gold™ kit (Zymo Research) as previously reported (Lubin et al. 2008). A fragment (about 250 bp) in the promoter region of *Cdk5* was amplified, independent of methylation status, by the primers as following: (5'-TTGGAAGGTTGTGAGTATAGAAAAG-3', 5'-CCATATTTCCCAAAATTACCTATAAC-3'). The PCR product was then purified using a gel extraction kit (Qiagen) and sequenced using the reverse primer at the institutional core facility. The percentage methylation of each CpG site within the region amplified was determined by the ratio between peaks values of guanine (G) and adenine (A) ($G/[G + A]$), and these levels on the electropherogram were determined using Chromas software.

Protein extraction and immunoblotting

The protein extraction and immunoblotting was performed in the hippocampal CA1 tissues (Sabatino et al.

2012). The tissues were collected from the rats immediately after the behavioral tests and lysed in ice-cold lysis buffer containing 55 mM Tris-Cl, 145 mM NaCl, 0.01 mM NaN₃, 100 µg/ml phenylmethylsulfonyl fluoride, 1 µg/ml aprotinin, 1 % Triton X-100 and proteinase inhibitor cocktail. Total protein was extracted and separated with SDS–polyacrylamide gel electrophoresis followed by blotting to a nitrocellulose membrane. The primary antibodies included polyclonal antibodies against Cdk5 (1:1000; Millipore), tau (1:1000; Santa Cruz Biotechnology) and phosphorylated Tau (1:1000; Santa Cruz Biotechnology). Appropriate horseradish peroxidase-conjugated secondary antibodies (1:10,000; Jackson ImmunoResearch Laboratories Inc.) were then applied, and the immunoreactivity was detected using enhanced chemiluminescence (ECL Advance Kit; Amersham Biosciences). The intensity of the bands was captured digitally and analyzed quantitatively with Image Lab software and compared among the appropriate groups.

Hippocampal slice preparation and whole-cell recordings

Hippocampal slices were prepared, and the whole-cell recording was performed on the CA1 neurons as previously described (Pita-Almenar et al. 2012). Immediately after the behavioral tests, the rats were deeply anesthetized with inhalation of halothane, and the brain was removed quickly. The coronal brain slices containing hippocampal CA1 were cut with a vibratome (Technical Products International, St. Louis, MO) and incubated in Krebs solution (containing: 117 mM NaCl, 3.6 mM KCl, 1.2 mM MgCl₂, 2.5 mM CaCl₂, 1.2 mM NaH₂PO₄, 11 mM glucose, and 24 mM NaHCO₃, bubbled with 95 % O₂ and 5 % CO₂) at 34 °C for at least 1 h before the recording was performed.

Visualized whole-cell voltage-clamp recordings were performed using an Axopatch700B amplifier (Molecular Devices) with 2–4 MΩ glass electrodes containing the following internal solution (in mM): cesium gluconate, 125; NaCl, 5; MgCl₂ 1.0; EGTA, 0.5; Mg-ATP, 2; Na₃GTP, 0.1; HEPES, 10; guanosine 5-*O*-(2-thiodiphosphate) 1; lidocaine *N*-ethyl bromide (QX314), 10; pH 7.3; 290–300 mOsmol. A seal resistance of ≥2 GΩ and an access resistance of 15–20 MΩ were considered acceptable. The series resistance was optimally compensated by ≥70 % and constantly monitored throughout the experiments. The membrane potential was held at –60 mV throughout the experiment. Schaffer collateral–commissural fibers were stimulated by concentric bipolar electrode, and the excitatory postsynaptic currents (EPSCs) were recorded in the CA1 neurons in the presence of bicuculline (30 µM). The evoked EPSCs were filtered at 2 kHz, digitized at 10 kHz, and acquired and analyzed using pCLAMP 9.2 software (Molecular Devices). Long-term potentiation (LTP) was

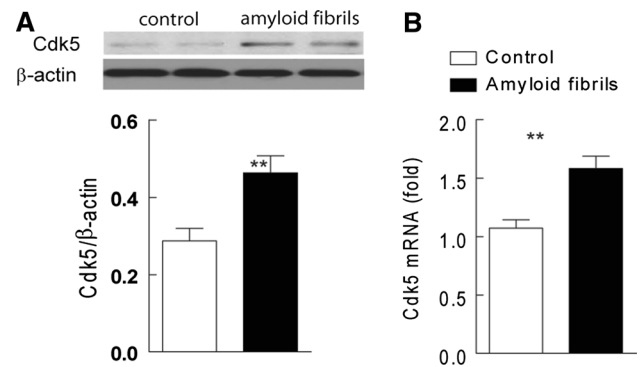


Fig. 1 Amyloid fibrils increased the expression of Cdk5 in hippocampal CA1. **a** Significantly increased expression of Cdk5 was observed in the hippocampal CA1 tissue in the rats injected with amyloid fibrils ($n = 7$ rats per group). **b** Significantly increased mRNA level of Cdk5 was observed in the hippocampal CA1 tissue in the rats injected with amyloid fibrils ($n = 7$ rats per group). * $P < 0.05$; ** $P < 0.01$

induced by a single high-frequency electric stimuli train (HFS, 100 Hz for 1 s) (Shipton et al. 2011). All electrophysiological experiments were performed at room temperature.

Drugs and data analysis

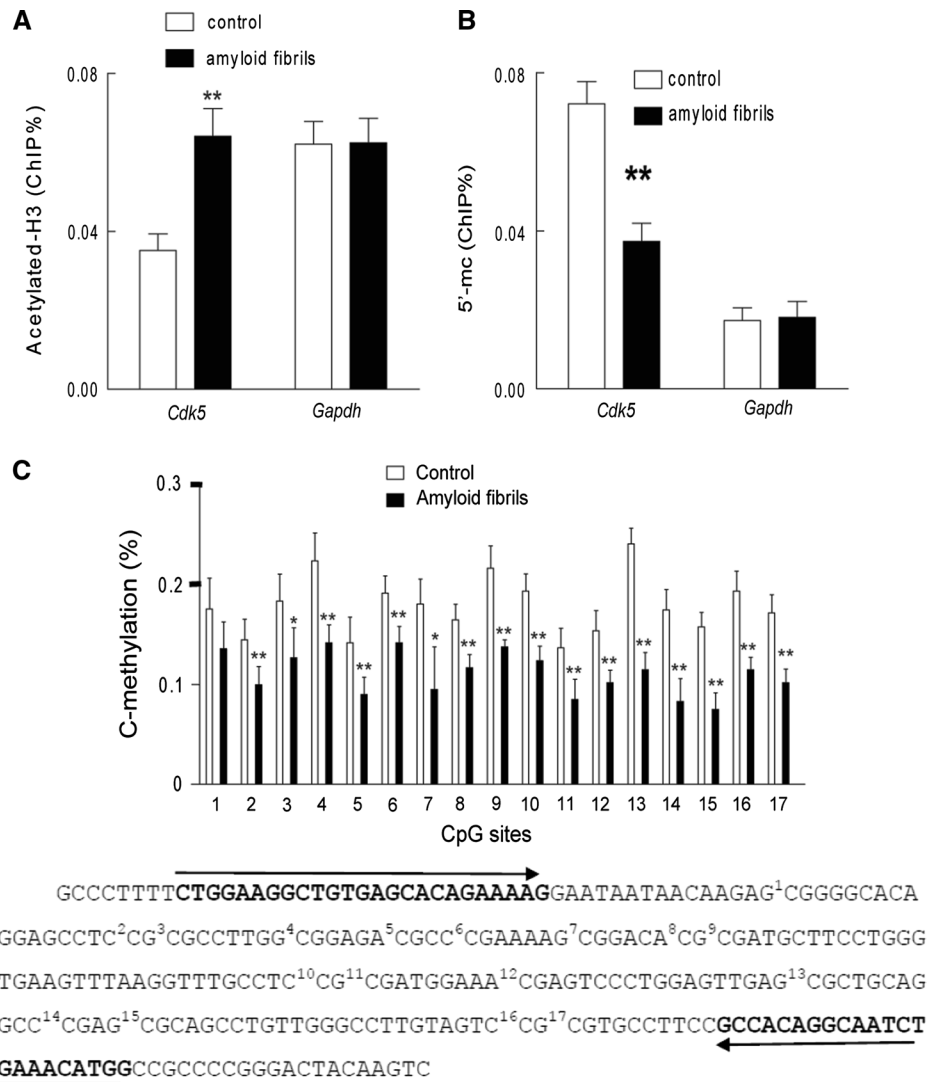
All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) or other commercial resource. All data were presented as mean \pm SEM, and analyzed with Student's *t* test and one- or two-way ANOVA followed by post hoc analysis. The criterion for statistical significance was $P < 0.05$.

Result

Increased hippocampal Cdk5 expression in the rats injected amyloid fibrils

Previous evidences indicated the critical role of dysregulation of Cdk5 activity in the pathophysiology of several neurodegenerative diseases (Cheung and Ip 2012). Then, we first detect the expression of Cdk5 in the hippocampal CA1 in the rats injected with amyloid fibrils. The immunoblotting results showed a significantly increased expression of Cdk5 in the hippocampal CA1 of the rats injected with amyloid fibrils (Fig. 1a, $n = 7$ in each group, $t = 3.49$, $P < 0.01$). The further reverse transcription real-time PCR analysis also revealed a significant increased level of Cdk5 mRNA in the hippocampal CA1 of the rats injected with amyloid fibrils (Fig. 1b, $n = 7$ in each group, $t = 3.64$, $P < 0.01$). These results demonstrated a substantial upregulation of hippocampal Cdk5 induced by amyloid fibrils,

Fig. 2 Amyloid fibrils altered histone H3 acetylation and cytosine methylation in *Cdk5* promoter region. **a** In ChIP study with polyclonal antibody against acetylated histone H3, significantly increased histone H3 acetylation in the promoter region of *Cdk5*, but not *Gapdh*, was detected in the hippocampal CA1 in the rats injected with amyloid fibrils ($n = 7$ per group). **b** In ChIP study with polyclonal antibody against 5'-methylcytosine, significantly decreased 5'-methylcytosine in the promoter region of *Cdk5*, but not *Gapdh*, was detected in the hippocampal CA1 in the rats injected with amyloid fibrils ($n = 7$ per group). **c** The change of methylation in the specific CpG sites in the *Cdk5* promoter region in the hippocampal CA1 was illustrated in the rats injected with amyloid fibrils ($n = 6$ per group). * $P < 0.05$; ** $P < 0.01$



which possibly underlay the memory impairment in the modeled rats.

Epigenetic modification in *Cdk5* promoter region

Increasing studies showed that epigenetic modification of histone acetylation and/or cytosine methylation substantially modulated the chromatin remodeling, thus regulating the transcription of target genes. Hence, we next studied the extent of histone H3 acetylation and cytosine methylation in the promoter region of *Cdk5* in the rats injected with amyloid fibrils. The ChIP studies with polyclonal antibody against acetylated histone H3 revealed significantly increased histone H3 acetylation in the promoter region of *Cdk5*, but not *Gapdh*, in the hippocampal CA1 in the rats injected with amyloid fibrils (Fig. 2a, $n = 7$ in each group, $t = 3.41$, $P < 0.01$). Meanwhile, with the polyclonal antibody against 5'-cytosine, further ChIP study also revealed significantly decreased cytosine methylation

in the promoter region of *Cdk5*, but not *Gapdh*, in the hippocampal CA1 in the modeled rats (Fig. 2b, $n = 7$ in each group, $t = 4.02$, $P < 0.01$). Further bisulfite sequencing PCR was performed to illustrate the change of methylation in the specific CpG sites in the *Cdk5* promoter region in the hippocampal CA1 in the rats injected with amyloid fibrils (Fig. 2c, $n = 6$ in each group). These results implied that the significant epigenetic modification in *Cdk5* promoter region potentially led to the upregulation of *Cdk5* in the hippocampal CA1 in the rats injected with amyloid fibrils.

Inhibition of *Cdk5* activity decreased Tau phosphorylation

Next, in order to explore the functional significance of upregulation of hippocampal *Cdk5*, the *Cdk5* inhibitor roscovitine ($5 \mu\text{g}$ for 3 days) was delivered into the hippocampal CA1 area in the rats previously injected with amyloid fibrils. As shown in Fig. 3, significantly increased expression of phosphorylated tau, but not total tau, was observed

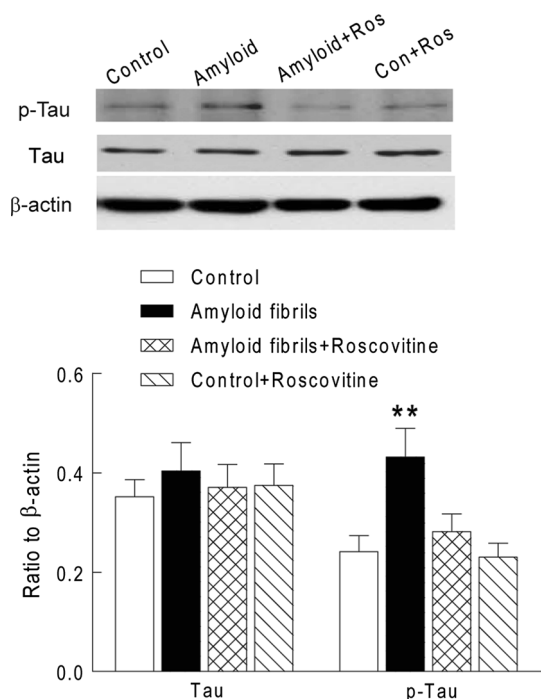


Fig. 3 Inhibition of Cdk5 activity decreased Tau phosphorylation. Significantly increased expression of phosphorylated tau, but not total tau, was observed in the hippocampal CA1 in the rats injected with amyloid fibrils, which was significantly attenuated by the treatment with roscovitine (5 μg for 3 days). $n = 7$ per group; * $P < 0.05$; ** $P < 0.01$

in the hippocampal CA1 in the rats injected with amyloid fibrils ($n = 7$ in each group, $t = 3.46$, $P < 0.01$), which was significantly attenuated by the treatment with roscovitine ($n = 7$ in each group, $t = 3.51$, $P < 0.01$). Note that treatment with roscovitine did not appreciably change the phosphorylated tau in the hippocampal CA1 in the control rats. These results indicated that inhibition of Cdk5 activity substantially reversed the phosphorylation of Tau in hippocampal CA1 in the rats injected with amyloid fibrils.

Inhibition of Cdk5 recovers synaptic dysfunction and memory deficiency induced by amyloid fibrils

We then performed whole-cell recording in hippocampal CA1 slices to explore the effect of inhibition of Cdk5 activity by roscovitine on the hippocampal synaptic plasticity in the rats injected with amyloid fibrils. As shown in Fig. 4, significantly impaired HFS-induced LTP was observed in the hippocampal CA1 neurons in the rats injected with amyloid fibrils ($n = 10$ and 11 neurons, $F_{(1,19)} = 10.78$, $P < 0.01$), and treatment with roscovitine significantly attenuated the synaptic impairment induced by amyloid fibrils ($n = 11$ and 10 neurons, $F_{(1,19)} = 9.84$, $P < 0.01$). It was also found that inhibition of Cdk5 activity

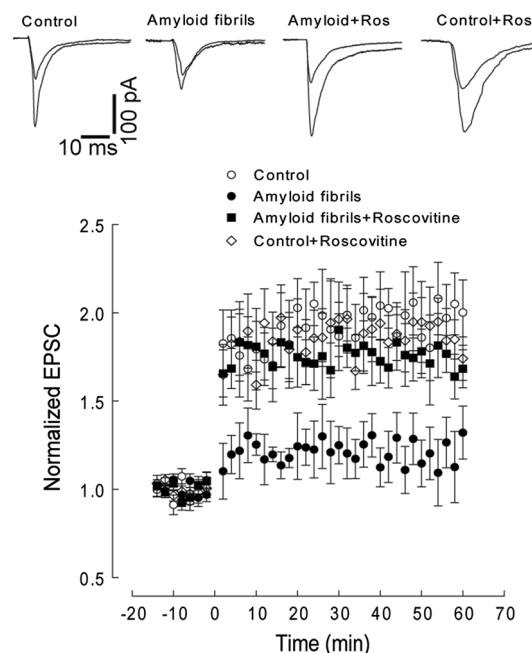


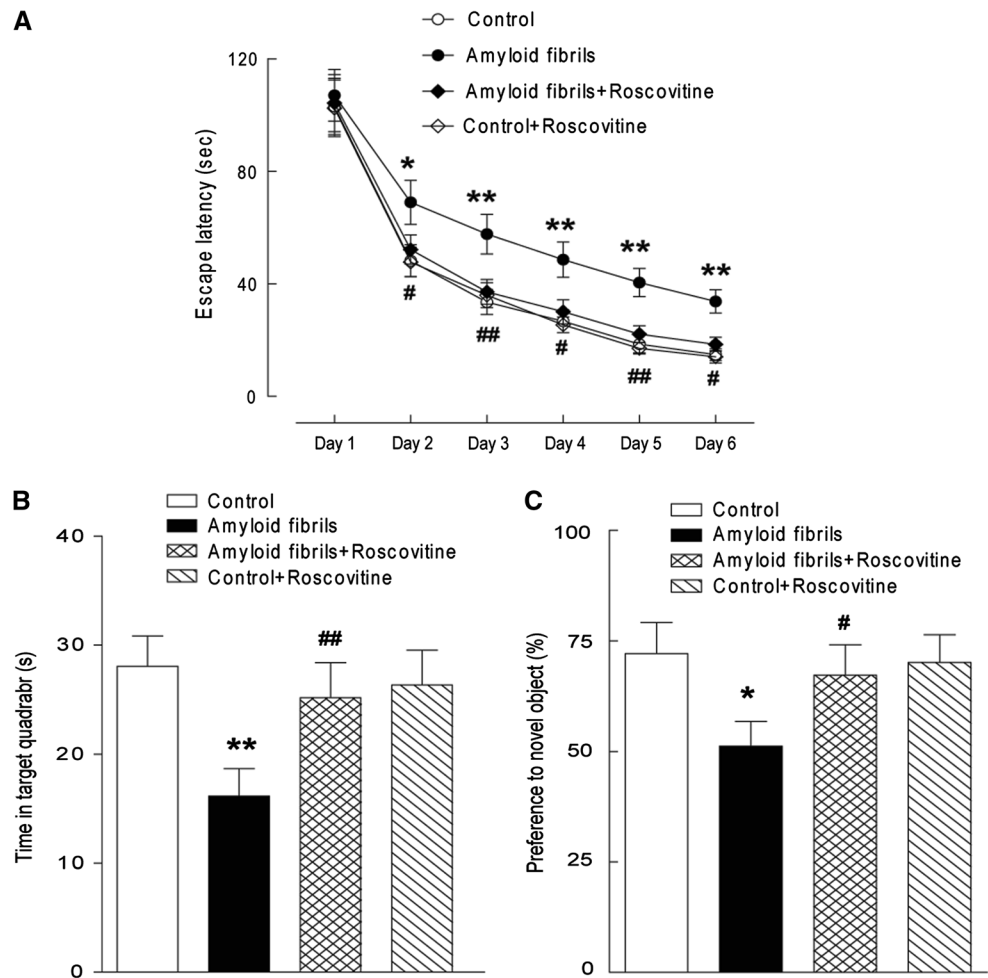
Fig. 4 Inhibition of Cdk5 activity recovered the synaptic plasticity impaired by amyloid fibrils. Significantly impaired HFS-induced LTP was observed in the hippocampal CA1 neurons in the rats injected with amyloid fibrils, which was significantly mitigated by the injection of roscovitine (5 μg for 3 days). $N = 9$ –11 neurons in each group

by roscovitine did not remarkably change the HFS-induced LTP in the hippocampal CA1 neurons in the control rats ($n = 10$ and 10 neurons, $F_{(1,18)} = 3.64$, $P > 0.05$).

Morris water maze testing was then performed to explore the effect of inhibition of Cdk5 activity by roscovitine on the spatial memory in the rats injected with amyloid fibrils. As shown in Fig. 5a, bilateral microinjection of amyloid fibrils (20 μg per side) into the hippocampal CA1 area significantly extended the escape latency in the Morris water maze test in the rats ($n = 10$ and 11 rats, $F_{(1,19)} = 10.74$, $P < 0.01$), which indicated a deficiency of memory acquisition. The rats injected with amyloid fibrils also spent less time in the target quadrant in the probe trail (Fig. 5b, $n = 10$ and 11 rats, $t = 3.51$, $P < 0.01$). It was also found that treatment with roscovitine significantly decreased the escape latency in the Morris water maze test (Fig. 5a, $n = 11$ and 12 rats in each group, $F_{(1,21)} = 9.91$, $P < 0.01$) and increased the time spent in the target quadrant in the probe trail (Fig. 5b, $n = 11$ and 12 rats in each group, $t = 3.17$, $P < 0.01$) in the rats injected with amyloid fibrils. Note that treatment with roscovitine did not induce significant change of escape latency and time spent in target quadrant in the control rats (Fig. 5a, b).

Consistently, the rats injected with amyloid fibrils exhibited significantly less preference to novel object in the novel object recognition test ($n = 10$ and 11 rats, $t = 2.39$, $P < 0.05$), which indicated an impaired short-term memory.

Fig. 5 Inhibition of Cdk5 activity recovered the memory deficiency induced by amyloid fibrils. **a** Microinjection of amyloid fibrils significantly extended the escape latency in the rats, which was significantly mitigated by the injection of roscovitine (5 μ g for 3 days) in the modeled rats. **b** The rats injected with amyloid fibrils spent less time in the target quadrant, which was significantly recovered by microinjection of roscovitine. **c** The rats injected with amyloid fibrils spent significantly less time to explore the novel object, which was significantly recovered by the microinjection of roscovitine. *N* = 9–11 rats per group. Compared to control group: **P* < 0.05; ***P* < 0.01, compared to amyloid fibrils group: #*P* < 0.05; ##*P* < 0.01



Treatment with roscovitine significantly increased the time exploring the novel object in the rats previously injected with amyloid fibrils (Fig. 5c, *n* = 11 and 12 rats, *t* = 2.33, *P* < 0.05), while it did not induce obvious change of preference to the novel object in the control rats (*n* = 10 rats per group, *t* = 0.52, *P* > 0.05). Note that there was no significance difference in the total exploring time among all groups. These results suggested the inhibition of Cdk5 activity by roscovitine substantially recovered the cognitive function impaired by the amyloid fibrils in the rats.

Discussion

Significant modification (e.g., acetylation, methylation or phosphorylation) of the histone or cytosine in the specific genomic regions may generally alter the chromatin remodeling and the expression of target genes (Rudenko and Tsai 2014). Altered histone acetylation and/or cytosine methylation in the promoter region remarkably modified the transcription of specific genes (e.g., *Bdnf*) pertinent to the memory formation and consolidation, thus contributing to

the impaired hippocampal synaptic plasticity and cognitive function in the rodent models of the AD and other neurodegenerative disorders (Guan et al. 2009; van den Hove et al. 2014). It was previously reported that significantly increased methylation of 5'-cytosine was correlated with the progression of amyloid beta and tau associated neurofibrillary pathology in several brain regions of the AD patients (Coppieters et al. 2014). Overexpression of histone deacetylase 2 (HDAC2), which reduced the histone acetylation and expression of genes important for learning and memory, was observed in the hippocampal CA1 area, and inhibition of HDAC2 activity substantially rescued the hippocampal dendritic spines and synaptic plasticity, and recovered the cognition function in the transgenic mice model of AD (Graff et al. 2012; Guan et al. 2009; Sung et al. 2013). The present study demonstrated another mechanistic scenario involving epigenetic modifications contributing to the pathogenesis of AD in the rodent model. Here, we found significant increase of acetylated histone H3 acetylation and decrease of methylated cytosine in the promoter region of *Cdk5*. These epigenetic alterations generally facilitated the accessibility of transcriptional machinery to

promoter region, which leading to the upregulated expression of target gene. Hence, these results illustrated the potential epigenetic mechanism underlying the upregulation of hippocampal Cdk5 in the rodent model of AD.

Increasing evidences demonstrated that several epigenetic mechanisms were involved in the modulation of expression and activity of Cdk5 in several neurological scenarios. Histone acetyltransferase GCN5 mediated the histone acetylation in the promoter region of *Cdk5*, thus facilitating the expression and activity of Cdk5 in the cultured HEK293 cells (Lee et al. 2014). Upregulated activity of histone acetyltransferases was associated with the ethanol-enhanced expression of Cdk5 in prefrontal cortex (Pascual et al. 2012). In the present study, significantly increased acetylation of histone H3 was observed in the promoter region of Cdk5 in the hippocampal CA1 in rats injected with amyloid fibrils. Further chromatin immunoprecipitation and bisulfite sequencing studies also illustrated a significantly decreased cytosine methylation in the hippocampal CA1 in the rats receiving amyloid fibrils. Generally, increased histone acetylation and decreased cytosine methylation were associated with the transition from heterochromatin to euchromatin, which facilitating the accessibility of transcriptional machinery in the targeted DNA sequence. Hence, the observed chromatin modifications, including histone acetylation and cytosine demethylation, in the *Cdk5* promoter region potentially contributed to the upregulation of hippocampal Cdk5 in the rats injected with amyloid fibrils.

Numerous evidences demonstrated the critical involvement of Cdk5 activity in the pathogenesis of amyloid-associated memory deficiency. Significant elevation in cdk5 specific kinase activity was found in the human AD brains (Lee et al. 1999) and rodent models of AD (Cheung et al. 2006). Prevailing evidences indicated that Cdk5 may physically interact with tau (Sobue et al. 2000) and phosphorylate several epitopes of tau, thus leading to the transformation of tau into neurofibrillary tangles (Flaherty et al. 2000). Upregulation of Cdk5 activity with its activator p25 also induced significant neuronal loss in the cerebral cortex in the transgenic mice (Mapelli et al. 2005). In addition, Cdk5 may regulate the activity of several postsynaptic scaffold proteins, including PSD95 (Colledge et al. 2003) and GKAP (Roselli et al. 2011), thus contributing to the impaired synaptic plasticity in the presence of amyloid fibrils (Cheung and Ip 2012; Lai et al. 2012). In the present study, significantly increased expression of Cdk5 was observed in the hippocampal CA1, and inhibition of Cdk5 activity by roscovitine substantially recovered the tau phosphorylation, hippocampal synaptic dysfunction and memory deficiency in the rats injected with amyloid fibrils. These findings collectively confirmed the critical role of Cdk5 activity in the pathophysiology of neuronal dysfunction and memory deficiency induced by amyloid fibrils.

Conclusion

The present study revealed a significant increase of hippocampal Cdk5, potentially resulting from the increased histone H3 acetylation and decreased cytosine methylation in the promoter region of *Cdk5*, in the rat model of AD. Inhibition of Cdk5 activity remarkably attenuated the phosphorylation of tau and mitigated the synaptic dysfunction and memory deficiency in the modeled rats. These may provided novel insights on the developing novel therapeutic approaches for the patients with amyloid-associated memory deficiency.

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