Neurochemical Research (2019) 44:1593–1601
https://doi.org/10.1007/s11064-019-02783-8

ORIGINAL PAPER

Chronic Intermittent Ethanol Exposure Induces Upregulation of Matrix Metalloproteinase-9 in the Rat Medial Prefrontal Cortex and Hippocampus

Litian Yin[1] · Fengqing Li[1] · Jue Li[2] · Xiaorong Yang[1] · Xiaoyan Xie[1] · Linyuan Xue[1] · Yanli Li[1] · Ce Zhang[1]

Received: 7 November 2018 / Revised: 15 March 2019 / Accepted: 18 March 2019 / Published online: 26 March 2019

Abstract

Matrix metalloproteinase-9 (MMP-9, Gelatinase B), an extracellular-acting Zn^{2+}-dependent endopeptidase, are involved in brain pathologies including ischemia, glioma, and epilepsy. Recent studies suggested that MMP-9 plays an important role in neuronal plasticity, specifically in learning and memory. To determine whether and how MMP-9 plays role in alcohol-related behaviors, male Sprague–Dawley (SD) rats were subjected to chronic intermittent ethanol (CIE) exposure for 4 weeks, following which we collected tissue samples from the hippocampus, medial prefrontal cortex (mPFC), and amygdala at different stages (acute and chronic exposure) during alcohol exposure. Real-time PCR and western blot assays were used to detect changes in the mRNA and protein expression of MMP-9. Our results indicated that both acute and chronic alcohol exposure induced up-regulation of MMP-9 mRNA levels in the hippocampus and mPFC, but not in the amygdala. Furthermore, acute and chronic alcohol exposure up regulated the expression of total MMP-9 and active MMP-9 in these two brain regions. Moreover, the increase of active MMP-9 expression was larger than those in total MMP-9 expression. Immunoprecipitation analyses identified potential MMP-9-interacting proteins, including Itgb1, Src, Eef1a2, tubulin, actin, and histone H2B. These results demonstrate that both acute and CIE exposure induced increases in MMP-9 expression in the mPFC and hippocampus, suggesting that MMP-9 plays a key role in chronic alcohol exposure and dependence.

Keywords MMP-9 · Chronic intermittent ethanol exposure · Hippocampus · Protein expression · Protein interaction

Introduction

Alcohol abuse and alcoholism interferes with alcoholics' personal and social life, causing a significant public health concern [1, 2]. The development of drug or alcohol addiction often involves the dysfunction of normal learning and memory circuits within the brain [3–5]. These are complex processes associated with the reformation of synaptic structures in specific brain regions that occur in conjunction with changes in signaling at the level of individual synapses (i.e., synaptic plasticity). Despite substantial efforts dedicated to understanding the regulation of drug-evoked plasticity within the neurotransmitter system, the underlying molecular mechanisms remained largely unknown [6]. However, recent evidence has implicated both genetic and epigenetic mechanisms in the control of synaptic plasticity in individuals with alcohol addiction [7–11].

Matrix metalloproteinases (MMPs), a family of zinc-dependent enzymes, can degrade components of the extracellular matrix (ECM) [12]. In particular, MMP-9 (Gelatinase B) binds integrin, thereby activating signaling involving cell adhesion molecules and pro-forms of growth factors [13]. Recently, MMP-9 has emerged as a novel regulator of physiological processes in the adult central nervous system (CNS), such as synaptic plasticity, learning, and memory [14, 15]. Moreover, previous studies have indicated that MMP-9 displayed enzymatic activity at synapses, helping

Litian Yin and Fengqing Li have contributed equally to this work.

✉ Litian Yin
yinlitian@126.com

✉ Ce Zhang
cezh2002@yahoo.com

[1] Key Laboratory for Cellular Physiology of Ministry of Education, Department of Physiology, National Key Disciplines, Shanxi Medical University, Taiyuan 030001, Shanxi, China

[2] School of Clinic, Shanxi Medical University, Taiyuan 030001, Shanxi, China

to establish persistent modifications by influencing dendritic spine enlargement and coordinating long-term potentiation (LTP) [5, 16, 17]. However, the role of MMP-9 in alcohol addiction remains unclear.

The mechanisms involved in alcohol exposure and withdrawal differ from those associated with the formation of addiction. In the present study, we aimed to identify the role and characteristics of MMP-9 at different stages during alcohol exposure to address whether and how MMP-9 influence alcohol related behaviors under alcohol exposure, and may aid comprehension the development of novel strategies for the treatment of alcohol dependence [18].

Materials and Methods

Experimental Animals

A total of 105 male Sprague–Dawley rats, aged 7 weeks, were purchased from the Laboratory Animal Center of the Academy of Military Medical Science (Beijing, China). The rats were housed in a temperature-controlled (22 °C) cage under a 12 h/12 h light/dark cycle. Food and water were provided *ad libitum*. All animal care and experimental procedures were approved by the Shanxi Medical University Institutional Animal Care and Use Committee (Permit Number: 20170320-1) and were conducted in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals*.

Chronic Intermittent Ethanol Treatment

CIE exposure was conducted as previously described [19], with minor modifications. Briefly, all rats were acclimated for 1 week prior to any experiments. The animals were randomly allocated into three groups: (1) Ethanol (EtOH) ($n=35$): Animals were administered 25% EtOH solution (4 g/kg) via gavage; (2) Maltose ($n=35$): Instead of EtOH gavaging, animals were treated with maltose solution (7 g/kg) at a dose that was isocaloric to that used in the EtOH group [20]; and (3) Chow ($n=35$): Animals were allowed free access to food and water without gavaging. The gavaging pattern was as follows: Each set of two consecutive EtOH exposure days was followed by a 2-consecutive-day temporary withdrawal period, resulting in a total of seven on/off cycles between Day 0 and Day 28. The same schedule was used for maltose animals (Fig. 1). Animals were sacrificed at Day 0, Day 2, Day 4, Day 14, Day 16, Day 26, and Day 28 ($n=5$ at each time point in each group) to collect tissues from hippocampus, mPFC, amygdala and serum. Samples were used to detect mRNA and protein expression level ($n=4$/time point), blood EtOH concentration ($n=5$/time point) and IP-MS ($n=3$).

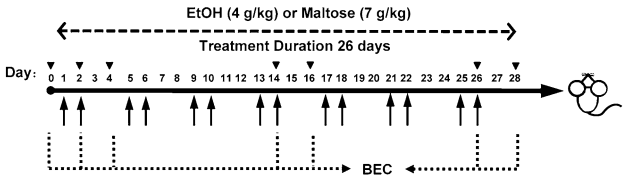

Fig. 1 CIE exposure protocol and experimental timeline. The schematic graph illustrates the protocol for CIE exposure. The arrowheads indicate the EtOH exposure periods, the inverted triangles indicate tissues collection time points, while the dotted lines indicate BEC collection time points. Animals were sacrificed for tissue collection at the end of Days 0 (baseline), Days 2 and 4 (initial), Days 14 and 16 (middle), and Days 26 and 28 (final). During the cessation of CIE, rats were weighed on each treatment dosing date. *CIE* chronic intermittent ethanol, *EtOH* ethanol, *BEC* blood ethanol concentration

Brian Collection

Rats were sacrificed via decollation under anesthesia with an overdose of pelltobarbitalum natricum at seven time-points (i.e., Day 0, 2, 4, 14, 16, 26, and 28), following which their brains were immediately extracted. The tissues from hippocampus, mPFC, and whole amygdala were dissected on an ice-cold platform in accordance with the rat brain atlas [21].

Reagents

Alcohol solutions were prepared using analytical grade reagents diluted to 25% (v/v) in tap water. Maltose monohydrate (Solarbio, China) solution was prepared by dissolving maltose monohydrate in tap water (5%, w/v).

Blood Ethanol Concentration (BEC)

Rats were gently restrained while the tip of the tail (around 3 mm) was cut off with a clean razor blade. Tail blood (0.2 ml) was collected and centrifuged to separate serum [22]. Blood samples were collected from rats in the EtOH group at Days 2, 14, and 26. The ethanol concentrations were immediately measured using gas chromatography [21].

Quantitative Real-Time PCR

Total RNA was extracted from hippocampus, prefrontal, and amygdala tissues using appropriate RNA extraction kits (Sangon, China). Synthesis of cDNA was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan), in accordance with the manufacturer's instructions. The resulting cDNA was used for quantitative real-time PCR. Thermal cycling was performed on a Step One Plus Real-Time PCR system (ABI, America), using a

relative calibration curve. GAPDH was used as the internal control, and PCR conditions for all primers were as follows: 30 s at 95 °C followed by 5 s at 95 °C, 34 s at 60 °C for 40 cycles. The following primers were designed using Primer3 software: *MMP-9*: upstream 5′-CTGTATGGTCGTGGCTCT AAAC-3′, reverse primer: 5′-GAGGTGCAGTGGGACACA TAG-3′; *GAPDH*: upstream 5′-GGAGAAACCTGCCAA GTATGA-3′, downstream 5′-TTGAAGTCACAGGAGACA ACC-3′.

Western Blot Analysis

Tissues were homogenized in ice-cold radio immunoprecipitation assay (RIPA) buffer (in mM: 25 Tris–HCl, pH 7.6, 150 NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with a proteinase inhibitor mixture containing 2 mM phenylmethylsulfonyl fluoride (PMSF), 20 µg/ml aprotinin, and 10 µg/ml leupeptin. Samples were homogenized using an ultrasonic crusher and centrifuged at 14000 × *g* for 30 min at 4 °C. The supernatants were collected, and the total proteins were assayed using the Bradford protein assay (Thermo Fisher, USA). Cell lysates were then subjected to SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, USA). Blots were blocked with 5% nonfat milk in PBS and then incubated with primary antibodies, anti-MMP-9 antibody (1/1000, Abcam, UK) and anti-GAPDH antibody (1/1000, Santa Cruz, USA), diluted in blocking solution overnight at 4 °C. The membranes were probed with HRP-conjugated second antibodies at room temperature for 1 h. Immunoreactive protein bands were visualized using a chemiluminescent substrate (Boster, China), in accordance with the manufacturer's instructions. Densitometry data were analyzed using a Gel Imaging System (Chemidoc MP, Bio-Rad, USA).

IP-MS Assay

Immunoprecipitation was performed as previously described [23]. Brain lysates were prepared and pre-cleared for 1 h at 4 °C with protein A/G agarose (Sigma Aldrich, USA). The lysates were incubated with anti-MMP-9 antibody (Abcam, USA). Immune complexes were collected after overnight incubation at 4 °C. After three washes with lysis buffer (with 0.2% NP-40), immune complexes were resolved via SDS-PAGE and transferred to nitrocellulose membranes (BioRad, USA). The LC-MS/MS analysis was performed as previously described [24], with minor modifications.

Statistical Analysis

All data are reported as the mean ± SEM for at least three independent experiments. Data were analyzed via one-way analyses of variance (ANOVA), followed by the LSD post *hoc test*. The level of statistical significance was set at $P < 0.05$. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, USA).

Results

Establishment of CIE Model

To characterize our CIE model, BECs were measured immediately following each treatment [25] (i.e., after the initial gavage (Day 2), middle period (Day 14), and final period (Day 26) (Fig. 2a). Average BEC values in each group and with different treatment were well within the range of binge EtOH benchmarks (70 mg/dl) in EtOH groups [26] which indicate that the model of alcohol-dependent rats was successfully established. Throughout the treatment period, all animals gained weight, and there was no significant effect of CIE treatment on animal weight (Fig. 2b).

Effect of CIE Exposure on Levels of MMP-9 mRNA

To investigate the relationship of MMP-9 and CIE exposure at different stages, real-time PCR was used to measure levels of MMP-9 mRNA expression at seven time points: baseline (Day 0), acute exposure (Day 2), acute withdrawal (Day 4), chronic exposure (Days 14 and 26), and chronic

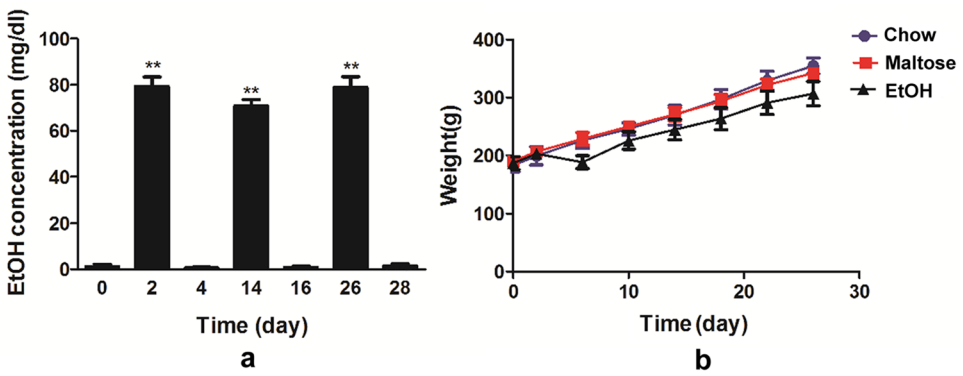

Fig. 2 Body weight and BEC during CIE exposure. **a** CIE induced pharmacologically relevant BECs. n = 5 rats per group; error bars indicate SEM. $^{**}P < 0.01$ versus chow. **b** Body weight from Days 0 through 28 in CIE rats. *BEC* blood ethanol concentration, *CIE* chronic intermittent ethanol, *EtOH* ethanol

withdrawal (Days 16 and 28). Acute ethanol exposure (Day 2) induced the increases of MMP-9 mRNA levels in the hippocampus ($F(2,9) = 10.627$, $P = 0.004$) and mPFC ($F(2,9) = 6.487$, $P = 0.018$). In short-term and long-term CIE periods (Days 14 and 26), MMP-9 mRNA levels also show significant increases in hippocampus ($F(2,9) = 8.828$, $P = 0.008$ and $F(2,9) = 21.391$, $P = 0.0003$, respectively) and mPFC ($F(2,9) = 4.36$, $P = 0.047$ and $F(2,9) = 4.536$, $P = 0.043$, respectively), while no such change was observed in the chow and maltose groups. We detected mRNA expression of MMP-9 during withdrawal periods as well. Acute ethanol withdrawal (Day 4) induced the increases of MMP-9 mRNA levels in the hippocampus ($F(2,9) = 10.150$, $P = 0.005$) and mPFC ($F(2,9) = 4.293$, $P = 0.049$). In short-term and long-term chronic ethanol withdrawal periods (Days 16 and 28), MMP-9 mRNA levels also show significant increases in hippocampus ($F(2,9) = 4.663$, $P = 0.041$ and $F(2,9) = 14.195$, $P = 0.002$, respectively) and mPFC ($F(2,9) = 6.744$, $P = 0.016$ and $F(2,9) = 5.365$, $P = 0.029$, respectively) (Fig. 3a, b). In addition, no significant change in MMP-9 mRNA level was observed in the amygdala during ethanol exposure (Fig. 3c).

We also detected the expression of the other collagenase IV, MMP-2 (Gelatinase A), in these regions. No significant change was observed in the hippocampus or mPFC following ethanol exposure or withdrawal. However, increases in MMP-2 expression were observed in the amygdala on Day 14 ($F(2,9) = 4.480$, $P = 0.045$) and Day 26 ($F(2,9) = 12.499$, $P = 0.003$) (Fig. 3d).

Chronic Alcohol Intake Increases the Translation of MMP-9 in the Hippocampus and mPFC

We then examined MMP-9 protein levels in the amygdala, hippocampus and mPFC in CIE rats at seven time points during the experiment. In the hippocampus, there was a significant increase of CIE treatment ($F(6,21) = 14.151$, $P < 0.0001$) on total MMP-9 level. The total MMP-9 were increased in the hippocampus after acute ethanol withdrawal (Day 4) ($F(6,21) = 14.151$, $P = 0.01$), and the maximal high level of total MMP-9 expression was observed at Day 28 ($F(6,21) = 14.151$, $P < 0.0001$), which increased MMP-9 expression by approximately threefold than that of Day 0. Meanwhile, active MMP-9 levels were higher during the CIE treatment than Day 0, which increased at Day 28 ($F(6,21) = 29.790$, $P < 0.0001$) by approximately 5.7-fold (Fig. 4a).

Changes in total MMP-9 levels were also observed in the medial prefrontal cortex. Compared with Day 0, the total MMP-9 levels began to increase during the early

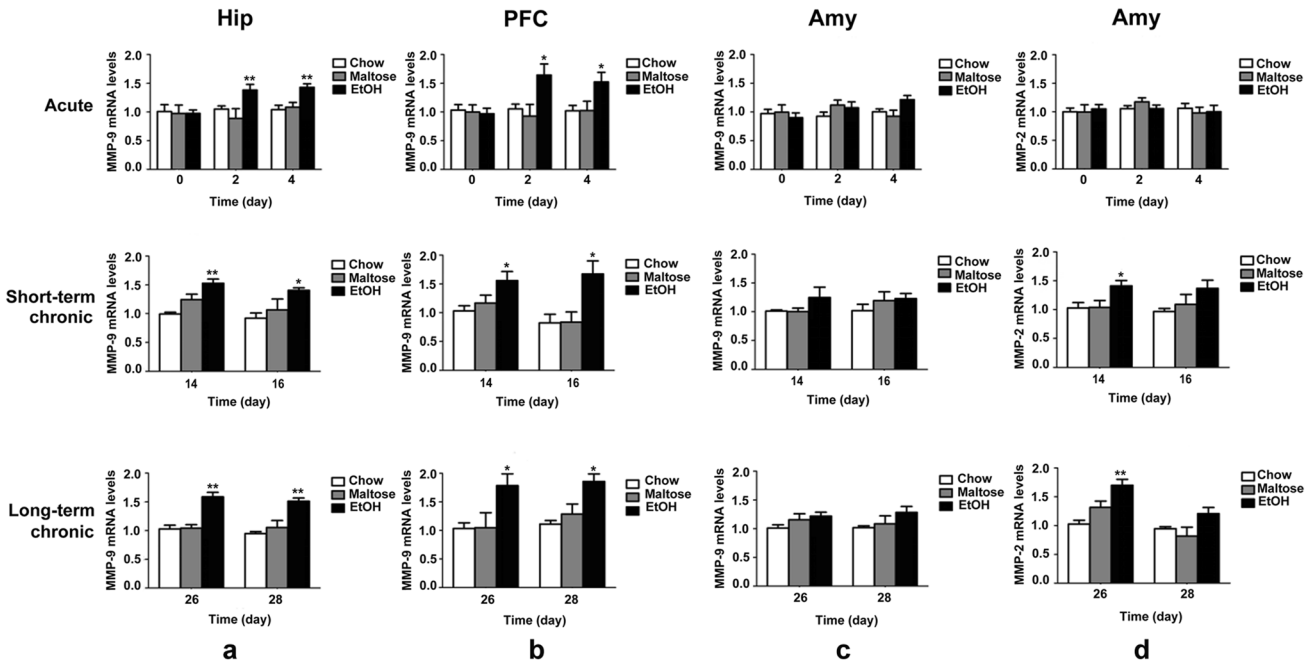

Fig. 3 Chronic ethanol exposure (CIE) induces MMP-9 mRNA expression in separated brain regions. Real-time PCR analysis for seven time points after CIE. **a** Expression of MMP-9 mRNA was observed in the hippocampus ($n = 4$/time point). $^{*}P < 0.05$ versus chow, $^{**}P < 0.01$ versus chow. **b** Expression of MMP-9 mRNA was observed in the medial prefrontal cortex. ($n = 4$/time point). $^{*}P < 0.05$ versus chow. **c** Expression of MMP-9 mRNA was observed in the amygdala. ($n = 4$/time point). **d** Expression of MMP-2 mRNA was observed in the amygdala. ($n = 4$/time point). $^{*}P < 0.05$ versus chow, $^{**}P < 0.01$ versus chow. *CIE* chronic intermittent ethanol, *PCR* polymerase chain reaction, *EtOH* ethanol, *MMP-9* matrix metalloproteinase-9

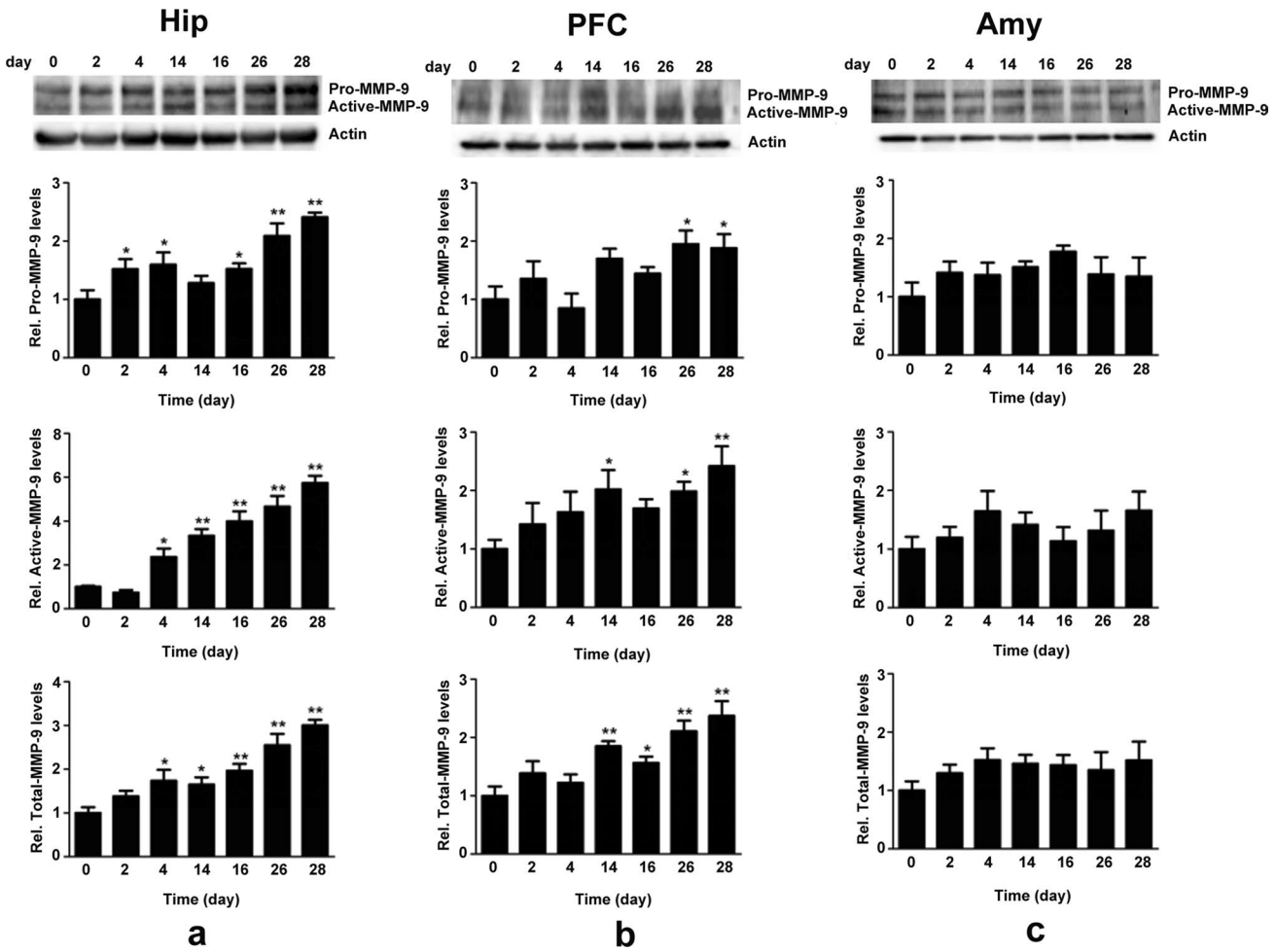

Fig. 4 Chronic ethanol exposure (CIE) induces MMP-9 protein expression in different brain regions. MMP-9 protein levels were tested at different stages of CIE treatment: baseline (Day 0), acute exposure (Day 2), acute withdrawal (Day 4), chronic exposure (Days 14 and 26) and chronic withdrawal (Days 16 and 28). **a** Pro- and active-MMP-9 protein expression at seven time points after CIE in the hippocampus ($n=4$/time point). $^{*}P<0.05$ versus Day 0, $^{**}P<0.01$ versus Day 0. **b** Pro- and active-MMP-9 protein expression at seven time points after CIE in the medial prefrontal cortex ($n=4$/time point). $^{*}P<0.05$ versus Day 0, $^{**}P<0.01$ versus Day 0. **c** Pro- and active-MMP-9 protein expression at seven time points after CIE in the amygdala ($n=4$/time point). *CIE* chronic intermittent ethanol, *MMP-9* matrix metalloproteinase-9

stages of chronic treatment (Day 14, $F(6,21)=8.298$, $P=0.002$ and Day 16, $P=0.029$), persisting to the later stages (Day 26, $F(6,21)=8.298$, $P<0.0001$ and Day 28, $P<0.0001$). In the mPFC, there were significant increases of active MMP-9 levels on Day 14 ($F(6,21)=2.631$, $P=0.019$), Day 26 ($F(6,21)=2.631$, $P=0.022$) and Day 28 ($F(6,21)=2.631$, $P=0.002$). On Day 28, the expressions of both active MMP-9 and total MMP-9 were increased by about 2.5-fold compared to Day 0 (Fig. 4b).

However, in the amygdala, we did not detect significant effects of CIE treatment on expression levels of total MMP-9 ($F(6,21)=0.683$, $P=0.666$), pro-MMP-9 ($F(6,21)=1.043$, $P=0.426$) and active MMP-9 ($F(6,21)=0.858$, $P=0.541$) compared with Day 0 (Fig. 4c).

These results indicated that MMP-9 levels were persistently elevated in the hippocampus and mPFC after acute, short-term chronic, and long-term chronic EtOH exposure, suggesting that such exposure was associated with stable changes in MMP-9 transcriptional regulation in these two brain regions. Changes were more evident for active MMP-9 (i.e., the functional state) than for total MMP-9 [27, 28].

Identification of Potential MMP-9-Binding Proteins Via LC-MS

As shown in Fig. 5, multiple potential MMP-9-interacting proteins co-immunoprecipitated with MMP-9 in the rat hippocampus on Day 28 in EtOH group, as indicated by the discrete silver-stained protein bands present in the

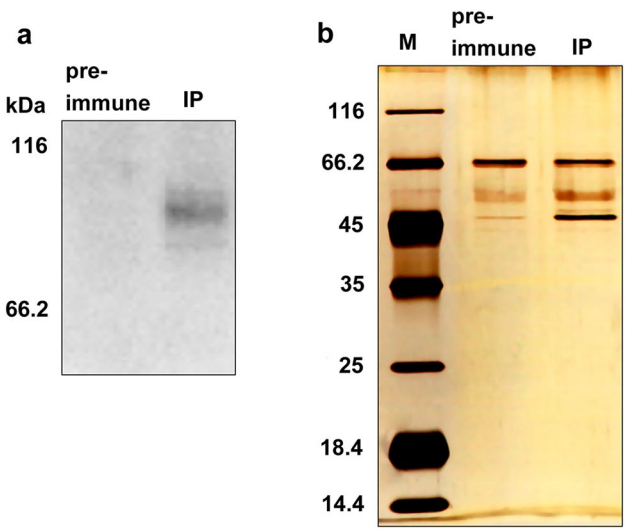

Fig. 5 Immunoprecipitation of MMP-9 and MMP-9-interacting proteins from the rat hippocampus. MMP-9 was immunoprecipitated from the rat hippocampus on Day 28 in EtOH group. **a** Following SDS-PAGE, the immunoprecipitates were analyzed via Western blot. MMP-9 immunoreactivity was detected in the MMP-9-immunoprecipitation (IP) lane but not in the IP lane containing rabbit pre-immune serum (pre-immune). **b**. Following SDS-PAGE, the immunoprecipitates were analyzed via silver-stained for total protein levels. Discrete protein bands were present in the MMP-9 IP lane but not in the IP lane containing rabbit pre-immune serum (pre-immune). *MMP-9* matrix metalloproteinase-9

Table 1 Potential MMP-9-interacting proteins

NCBI accession	Protein	No. of unique peptides	MASCOT score
P49134	Integrin subunit beta 1	6	158
Q9WUD9	SRC proto-oncogene	5	128
P62632	Elongation factor 1-alpha 2	6	204
P68370	Tubulin alpha-1A chain	11	297
A0A0G2K3K2	Actin, cytoplasmic 1	5	125
D3ZRN3	Actin, beta-like 2	7	213
A0A0G2JXI9	Histone H2B	2	145

MASCOT score: Proteins were identified using the Mascot search engine (version 2.2.1, Matrix Science, Inc., Boston, MA) based on the combined information of the accurate peptide molecular weight and the corresponding MS/MS sequence information

immunoprecipitation lane, which were absent in the pre-immune lane (Fig. 5b). Table 1 lists the proteins identified, which included Itgb1, Src, Eef1a2, tubulin, actin, and histone H2B, including the respective NCBI accession numbers for the identified proteins, the number of unique tryptic peptides identified for each protein, and the corresponding MASCOT scores.

Discussion

In the present study, we examined MMP-9 expression in the rat brain at the acute, short-term chronic, and long-term chronic periods during CIE exposure and discovered the increases of MMP-9 mRNA and protein expression in the hippocampus and mPFC, not in the amygdala. Furthermore, the active MMP-9, the functional type of MMP-9 [18] significantly increased, in the exceeding of the elevated level of total MMP-9 when compared with control.

Our findings indicated that both MMP-9 mRNA and protein expression were up-regulated during the acute treatment periods, however further increases were observed on the short-term and long-term chronic stages. These increases were not directly related to alcohol administration or withdrawal, suggesting that changes in MMP-9 expression was a continuous and long-term process [29, 30].

In the present study, we examined three brain regions that were associated with learning, memory, and the reward system. Consistent with the findings of previous studies, we observed MMP-9 expression in the normal adult rat brain [31]. Activity-dependent transcription, local translation, and release of MMP-9 are implicated in the morphological plasticity of mature circuits [16, 18, 30]. During the CIE experiment, expression of total MMP-9 increased in the hippocampus and mPFC, but not in the amygdala. This finding indicates that changes in MMP-9 expression may be induced by enhanced synaptic activity involving the limbic system [32]. Pro-MMP-9 is recruited to the cell surface, where it is transformed to active MMP-9 by membrane-bound MT-MMPs. Previous studies have indicated that this molecule was activated at or near the cell surface [33], potentially playing a role in synaptic plasticity [1, 32]. MMP-9 is enriched in the postsynaptic density, our findings did not indicate changes of MMP-9 in the amygdala, which may be due to the fact that we did not separate the synaptic part from the entire amygdala [34].

Rats CIE administration provides a valid model for studying acute or chronic alcohol exposure and withdrawal. Rat weight and BEC levels after 2 days of withdrawal were used to verify the CIE model in the present study. Moreover, no significant differences in MMP-9 mRNA levels were observed between the chow and maltose groups at any time point.

MMPs are Zn^{2+}-dependent proteolytic enzymes acting as primary regulators of the composition and integrity of the extracellular matrix. Previous studies have uncovered the roles of MMPs in angiogenesis, tumor cell invasion, wound healing [13], cell signaling, and tissue remodeling [29]. MMP-9 is expressed in adult brain structures such as

the hippocampus, cerebral cortex, and cerebellum. While it is mostly produced by neurons, MMP-9 is also produced to some extent in glia [18]. Recent studies strongly suggested that MMP-9 plays a functional role in synaptic remodeling. Such activity may explain the physiological roles of MMP-9 in LTP, learning and memory [29, 35, 36], and in pathological conditions associated with changes in neuronal plasticity [e.g., amyotrophic lateral sclerosis and drug addiction] [18, 32, 37].

Accumulating evidence suggests that MMP-9 plays a key role in alcohol preference and alcohol-seeking behavior. Dysregulation of MMP-9 activity has been reported following chronic use of alcohol, nicotine, opiates, cocaine, and methamphetamine [13]. Analysis of the 139 families, Caucasians, suggests a higher efficiency of MMP-9 system in alcohol dependence [38]. Recent research has further indicated that MMP-9-knockout mice exhibited decreased motivation for alcohol and impaired alcohol-seeking behavior during withdrawal. Such findings suggest that alcohol-seeking behavior involves MMP-9-dependent alterations in dendritic spines and the generation of silent synapses in the central nucleus of the amygdala (CeA), which may be associated with the motivation for alcohol in humans [6, 13, 36]. The results of our study may provide the first evidence for the involvement of the MMP-9 gene in alcohol dependence in a rat CIE model. In addition, our study was the first time to provide such evidence based on mRNA and protein expression in an animal model of alcohol dependence. We had measure the other member of type IV collagenase/gelatinase MMP-2, which reported has propinquity function associated with tumor aggressiveness and migration [39, 40]. Our results showed mRNA levels of MMP-2 was increased in amygdala, suggesting that MMP-2 proteolysis participates in synaptic plasticity in amygdala.

Recent studies have demonstrated that MMP-9 modulates the morphology of dendritic spines [32], suggesting that MMP-9 is associated with the regulation of functional plasticity, signal modulation, and transformation of dendritic spines at excitatory synapses. Chronic developmental binge-like EtOH exposure alters the dendritic morphology of mPFC pyramidal and hippocampal neurons in adult mice [41]. In addition, inhibition of MMP-9 may assist the process of spine elongation and promotes the transformation of dendritic spines toward mature, mushroom-shaped spines [18]. Previous studies have indicated that MMP-9 signaling through beta-1 integrin subunits controls NMDA receptor surface diffusion into synapses, providing a potential mechanism by which MMP-9 deletion reduces NMDA currents and silent synapse formation [11, 13]. The substrate repertoire of MMP-9 has been enlarged from extracellular to membrane-bound and efficient intracellular substrates, such as crystallins, tubulins, and actins [12]. Moreover, the underlying mechanisms may involve regulation via tissue inhibitors of metalloproteinases [35, 42] or epigenetic factors [1, 11] (i.e., methylation, acetylation, and miRNA) [4, 43, 44]. In the present study, we observed that MMP-9 interacted with Itgb1, Src, Eef1a2 [45], tubulin, actin, and histone H2B, in accordance with the findings of previous studies. Src and Eef1a2 were interrelated with cell adhesion, synaptic transmission and plasticity [46]; cytoskeleton protein tubulin and actin were participated in process of cell remodeling and dendritic spine motility [34]; it's worth mentioning that Itgb1, a classic interaction protein of MMP-9, which is required in MMP-9-driven plasticity [47]. These results indicated that the mechanism of MMP-9 mediated synaptic plasticity remodeling was associated with cell adhesion, cytoskeletal remodeling and integrin signaling. Taken together, these findings shed new light on the role of MMP-9 in alcohol addiction. In identifying MMP-9 and its interaction proteins, multiple approaches, including co-localization, reciprocal immunoprecipitation, are applied to prove the hypothesis. In future, the studies are necessary to further substantiate function of MMP-9 and its interaction proteins in the development of experimental alcohol dependence.

Conclusion

In the present study, we demonstrated that chronic alcohol exposure induces MMP-9 expression in the rat hippocampus and mPFC. In addition, we observed that MMP-9 activation was markedly increased compared to control. Furthermore, our IP-MS assay identified several potential MMP-9-interacting proteins, which serves as potential target to prevent the alcohol addiction. Taken together, these findings shed new light on the role of MMP-9 in alcohol addiction. Since MMP-9 may be involved in the activity-dependent regulation of the peridendritic environment, in turn influencing synaptic physiology, future studies should investigate the detailed mechanisms mediating MMP-9 expression in alcohol addiction.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant Nos. 81601167, 31000481), the Natural Science Foundation of Shanxi Province, China (Grant Nos. 2013021022-1, 2011011040-2), and the Fund for Shanxi "1331 Project" Key Subjects Construction (Grant No. 1331KSC).

Compliance with Ethical Standards

Conflict of interest All authors declare that there are no conflicts of interest.

Research Involving Human and Animal Participants All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Kyzar EJ, Pandey SC (2015) Molecular mechanisms of synaptic remodeling in alcoholism. Neurosci Lett 601:11–19
2. Shepard BD, Tuma PL (2009) Alcohol-induced protein hyperacetylation: mechanisms and consequences. World J Gastroenterol 15(10):1219
3. Márquez J, Campos-Sandoval JA, Peñalver A, Matés JM, Segura JA, Blanco E, Alonso FJ, de Fonseca FR (2017) Glutamate and brain glutaminases in drug addiction. Neurochem Res 42(3):846–857
4. Robison AJ, Nestler EJ (2011) Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci 12(11):623–637
5. Ponomarev I, Wang S, Zhang L, Harris RA, Mayfield RD (2012) Gene coexpression networks in human brain identify epigenetic modifications in alcohol dependence. J Neurosci 32(5):1884–1897
6. Stefaniuk M, Beroun A, Lebitko T, Markina O, Leski S, Meyza K, Grzywacz A, Samochowiec J, Samochowiec A, Radwanska K, Kaczmarek L (2017) Matrix metalloproteinase-9 and synaptic plasticity in the central amygdala in control of alcohol-seeking behavior. Biol Psychiatry 81(11):907–917
7. Qin L, Han YP (2010) Epigenetic repression of matrix metalloproteinases in myofibroblastic hepatic stellate cells through histone deacetylases 4: implication in tissue fibrosis. Am J Pathol 177(4):1915–1928
8. Estella C, Herrer I, Atkinson SP, Quiñonero A, Martínez S, Pellicer A, Simón C (2012) Inhibition of histone deacetylase activity in human endometrial stromal cells promotes extracellular matrix remodeling and limits embryo invasion. PLoS ONE 7(1):e30508
9. Parira T, Laverde A, Agudelo M (2017) Epigenetic interactions between alcohol and cannabinergic effects: focus on histone modification and DNA methylation. J Alcohol Drug Depend 5(2):259
10. Li J, Li J, Liu X, Qin S, Guan Y, Liu Y, Cheng Y, Chen X, Li W, Wang S, Xiong M, Kuzhikandathil EV, Ye JH, Zhang C (2013) MicroRNA expression profile and functional analysis reveal that miR-382 is a critical novel gene of alcohol addiction. EMBO Mol Med 5(9):1402–1414
11. Labrie M, St-Pierre Y (2013) Epigenetic regulation of mmp-9 gene expression. Cell Mol Life Sci 70(17):3109–3124
12. Dziembowska M, Wlodarczyk J (2012) MMP9: a novel function in synaptic plasticity. Int J Biochem Cell Biol 44(5):709–713
13. Smith ACW (2017) A glitch in the matrix: aberrant extracellular matrix proteolysis contributes to alcohol seeking. Biol Psychiatry 81(11):900–902
14. Rivera S, Khrestchatisky M, Kaczmarek L, Rosenberg GA, Jaworski DM (2010) Metzincin proteases and their inhibitors: foes or friends in nervous system physiology? J Neurosci 30(46):15337–15357
15. Liu WT, Han Y, Liu YP, Song AA, Barnes B, Song XJ (2010) Spinal matrix metalloproteinase-9 contributes to physical dependence on morphine in mice. J Neurosci 30(22):7613–7623
16. Wang XB, Bozdagi O, Nikitczuk JS, Zhai ZW, Zhou Q, Huntley GW (2008) Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. Proc Natl Acad Sci 105(49):19520–19525
17. Gawlak M, Gorkiewicz T, Gorlewicz A, Konopacki FA, Kaczmarek L, Wilczynski GM (2009) High resolution in situ zymography reveals matrix metalloproteinase activity at glutamatergic synapses. Neuroscience 158(1):167–176
18. Dziembowska M, Milek J, Janusz A, Rejmak E, Romanowska E, Gorkiewicz T, Tiron A, Bramham CR, Kaczmarek L (2012) Activity-dependent local translation of matrix metalloproteinase-9. J Neurosci 32(42):14538–14547
19. Fernandez GM, Lew BJ, Vedder LC, Savage LM (2017) Chronic intermittent ethanol exposure leads to alterations in brain-derived neurotrophic factor within the frontal cortex and impaired behavioral flexibility in both adolescent and adult rats. Neuroscience 348:324–334
20. Qiang MWM, Elberger AJ (2002) Second trimester prenatal alcohol exposure alters development of rat corpus callosum. Neurotoxicol Teratol 24(6):719–732
21. Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. Amsterdam, Boston
22. Somkuwar SS, Fannon MJ, Staples MC, Zamora-Martinez ER, Navarro AI, Kim A, Quigley JA, Edwards S, Mandyam CD (2016) Alcohol dependence-induced regulation of the proliferation and survival of adult brain progenitors is associated with altered BDNF-TrkB signaling. Brain Struct Func 221(9):4319–4335
23. Liu Y, Zhang J, Yi B, Chen M, Qi J, Yin Y, Lu X, Jasmin JF, Sun J (2014) Nur77 suppresses pulmonary artery smooth muscle cell proliferation through inhibition of the STAT3/Pim-1/NFAT pathway. Am J Respir Cell Mol Biol 50(2):379–388
24. Hadlock GC, Nelson CC, Baucum AJ II, Hanson GR, Fleckenstein AE (2011) Ex vivo identification of protein-protein interactions involving the dopamine transporter. J Neurosci Meth 196(2):303–307
25. Jung ME, Wilson AM, Ju X, Wen Y, Metzger DB, Simpkins JW (2009) Ethanol withdrawal provokes opening of the mitochondrial membrane permeability transition pore in an estrogen-preventable manner. J Pharmacol Exp Ther 328(3):692–698
26. Cagetti E, Pinna G, Guidotti A, Baicy K, Olsen RW (2004) Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. Neuropharmacology 46(4):570–579
27. Aluri HS, Kublin CL, Thotakura S, Armaos H, Samizadeh M, Hawley D, Thomas WM, Leavis P, Makarenkova HP, Zoukhri D (2015) Role of matrix metalloproteinases 2 and 9 in lacrimal gland disease in animal models of sjögren's syndrome. Invest Ophthalmol Vis Sci 56(9):5218–5228
28. Peñalver A, Campos-Sandoval JA, Blanco E, Cardona C, Castilla L, Martín-Rufián M, Estivill-Torrús G, Sánchez-Varo R, Alonso FJ, Pérez-Hernández M, Colado MI, Gutiérrez A, de Fonseca FR, Márquez J (2017) Glutaminase and MMP-9 downregulation in cortex and hippocampus of LPA1 receptor null mice correlate with altered dendritic spine plasticity. Front Mol Neurosci 5(10):278
29. Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, Huntley GW (2006) Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci 26(7):1923–1934
30. Murase S, Lantz CL, Kim E, Gupta N, Higgins R, Stopfer M, Hoffman DA, Quinlan EM (2016) Matrix metalloproteinase-9 regulates neuronal circuit development and excitability. Mol Neurobiol 53(5):3477–3493
31. Szklarczyk A, Lapinska J, Rylski M, McKay RD, Kaczmarek L (2002) Matrix metalloproteinase-9 undergoes expression and activation during dendritic remodeling in adult hippocampus. J Neurosci 22(3):920–930
32. Michaluk P, Wawrzyniak M, Alot P, Szczot M, Wyrembek P, Mercik K, Medvedev N, Wilczek E, De Roo M, Zuschratter W, Muller D, Wilczynski GM, Mozrzymas JW, Stewart MG, Kaczmarek L, Wlodarczyk J (2011) Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. J Cell Sci 124(Pt 19):3369–3380
33. Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 69(3):562–573
34. Laguesse S, Morisot N, Shin JH, Liu F, Adrover MF, Sakhai SA, Lopez MF, Phamluong K, Griffin WC III, Becker HC, Bender KJ,

Alvarez VA, Ron D (2017) Prosapip1-dependent synaptic adaptations in the nucleus accumbens drive alcohol intake, seeking, and reward. Neuron 96(1):145–159
35. Okulski P, Jay TM, Jaworski J, Duniec K, Dzwonek J, Konopacki FA, Wilczynski GM, Sánchez-Capelo A, Mallet J, Kaczmarek L (2007) TIMP-1 abolishes MMP-9-dependent long-lasting long-term potentiation in the prefrontal cortex. Biol Psychiatry 62(4):359–362
36. Bach DR, Tzovara A, Vunder J (2018) Blocking human fear memory with the matrix metalloproteinase inhibitor doxycycline. Mol Psychiatry 23(7):1584–1589
37. Morisaki Y, Niikura M, Watanabe M, Onishi K, Tanabe S, Moriwaki Y, Okuda T, Ohara S, Murayama S, Takao M, Uchida S, Yamanaka K, Misawa H (2016) Selective expression of osteopontin in ALS-resistant motor neurons is a critical determinant of late phase neurodegeneration mediated by matrix metalloproteinase-9. Sci Rep 6(6):27354
38. Samochowiec A, Grzywacz A, Kaczmarek L, Bienkowski P, Samochowiec J, Mierzejewski P, Preuss UW, Grochans E, Ciechanowicz A (2010) Functional polymorphism of matrix metalloproteinase-9 (MMP-9) gene in alcohol dependence: family and case control study. Brain Res 1327:103–106
39. Swetha R, Gayen C, Kumar D, Singh TD, Modi G, Singh SK (2018) Biomolecular basis of matrix metallo proteinase-9 activity. Future Med Chem 10(9):1093–1112
40. Webb AH, Gao BT, Goldsmith ZK, Irvine AS, Saleh N, Lee RP, Lendermon JB, Bheemreddy R, Zhang Q, Brennan RC, Johnson D, Steinle JJ, Wilson MW, Morales-Tirado VM (2017) Inhibition of MMP-2 and MMP-9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma. BMC Cancer 17(1):434
41. Louth EL, Luctkar HD, Heney KA, Bailey CDC (2018) Developmental ethanol exposure alters the morphology of mouse prefrontal neurons in a layer-specific manner. Brain Res 1678:94–105
42. Arpino V, Brock M, Gill SE (2015) The role of TIMPs in regulation of extracellular matrix proteolysis. Matrix Biol 44–46:247–254
43. Nestler EJ (2005) Is there a common molecular pathway for addiction? Nat Neurosci 8(11):1445–1449
44. Tapocik JD, Barbier E, Flanigan M, Solomon M, Pincus A, Pilling A, Sun H, Schank JR, King C, Heilig M (2014) microRNA-206 in rat medial prefrontal cortex regulates BDNF expression and alcohol drinking. J Neurosci 34(13):4581–4588
45. Gkogkas CG, Khoutorsky A, Cao R, Jafarnejad SM, Prager-Khoutorsky M, Giannakas N, Kaminari A, Fragkouli A, Nader K, Price TJ, Konicek BW, Graff JR, Tzinia AK, Lacaille JC, Sonenberg N (2014) Pharmacogenetic inhibition of eIF4E-dependent MMP9 mRNA translation reverses fragile X syndrome-like phenotypes. Cell Rep 9(5):1742–1755
46. Lorraine VK, Jeffrey RG, Michael WS (2004) Src in synaptic transmission and plasticity. Oncogene 23:8007–8016
47. Michaluk P, Mikasova L, Groc L, Frischknecht R, Choquet D, Kaczmarek L (2009) Matrix metalloproteinase-9 controls NMDA receptor surface diffusion through integrin beta1 signaling. J Neurosci 29(18):6007–6012