ARTICLE OPEN

Check for updates

Chronic postoperative pain induces contextual fear extinction dysfunction through hippocampal NMDAR/BDNF/TrkB signaling pathway in mice

Jiawei Zhang[1,2,4], Xiaoting Zheng[3,4], Gaoyan Zhang[1,2], Zhikun Cheng[1,2], Yinuo Liu[1,2], Lei Zhang[1,2], Jiqian Zhang[1,2], Xuesheng Liu[1,2]✉ and Zhilai Yang[1,2]✉

Post-traumatic stress disorder (PTSD) is a common disorder in clinical practice, characterized by various manifestations, with fear extinction dysfunction being a typical one. Postoperative persistent pain, a form of chronic pain following surgical procedures, significantly affects patients' quality of life. Clinical studies have demonstrated the comorbidity between chronic pain and PTSD; however, the molecular mechanisms underlying this comorbidity remain unclear. Researches have shown that brain-derived neurotrophic factor (BDNF) and N-methyl-D-aspartate glutamate receptors (NMDARs) are crucial in fear extinction dysfunction. Thus, we established a skin/muscle incision and retraction (SMIR) mice model to explore the roles of hippocampal NMDARs and BDNF signaling pathways in fear extinction following postoperative persistent pain. We found that SMIR mice exhibited contextual fear extinction dysfunction, potentially caused by a down-regulated NMDARs/ERK/CREB/BDNF signaling pathway and impaired synaptic plasticity in the hippocampus. Hippocampal injection of the NMDARs agonist NMDA promoted extinction learning and retrieval of extinction memory, activating the NMDARs/ERK/CREB/BDNF signaling pathway, and restoring lost dendritic spines. Simultaneous hippocampal administration of NMDA and the TrkB inhibitor ANA-12 promoted the learning process of fear extinction without enhancing the retrieval of extinction memory, while re-inducing dendritic spine loss. In summary, we conclude that postoperative persistent pain impairs synaptic plasticity by downregulating the NMDARs/ERK/CREB/BDNF signaling pathway, thereby inducing contextual fear extinction dysfunction. These findings may partially explain the mechanisms underlying the comorbidity between chronic pain and PTSD.

Translational Psychiatry (2025)15:203 ; https://doi.org/10.1038/s41398-025-03417-0

INTRODUCTION

PTSD is classified into the following models based on pathophysiological research: abnormal fear learning model (FL), exaggerated threat detection model (TD), and emotion regulation/functional execution impairment model (EF). Fear extinction dysfunction, a typical manifestation of PTSD, has garnered increasing attention in recent years [1]. In Pavlovian fear conditioning experiments, fear extinction is defined as the inhibition of a conditioned fear response, which initially elicits a fear reflex (freezing) but gradually decreases with prolonged and repeated exposure to a conditioned stimulus (CS) in the absence of the unconditioned stimulus (US). When fear extinction dysfunction occurs, this conditioned fear suppression fails to manifest [2]. Extinction learning involves the acquisition of a memory that antagonizes the original fear memory, rather than forgetting it. This process competes with the previously learned fear memory and forms the basis of the widely used exposure therapy for fear memory [3, 4]. Context is broadly defined as a set of circumstances surrounding an event [5]. Increasing research focuses on the neural mechanisms that encode context in the brain. Neural circuits, including the hippocampus, medial prefrontal cortex, and amygdala, are involved in contextual learning and memory [5, 6]. The hippocampus plays a crucial role in contextual fear conditioning, with regions such as the dorsal hippocampus (dHPC) [7], hippocampal dentate gyrus (DG) [6], CA1 [8], and CA3 [9] being involved in the modulation of contextual fear memory extinction. The hippocampal CA1 area is considered a key region for accurate contextual memory [10]. Therefore, understanding the neurobiomolecular mechanisms related to fear extinction in the hippocampus is crucial for the treatment of PTSD.

Chronic pain affects 30% of the global population, significantly impairing patients' quality of life [11]. According to the biopsychosocial model, the development of chronic pain is associated with depression [12], anxiety [13], PTSD [14], and other psychological disorders, exhibiting significant comorbidity [11, 15, 16]. The comorbidity of chronic pain and mental illness is becoming a research hotspot in the field of pain [17, 18]. Clinical studies have shown that the incidence of PTSD among patients

[1]Department of Anesthesiology, the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui Province 230022, China. [2]Key Laboratory of Anesthesiology and Perioperative Medicine of Anhui Higher Education Institutes, Anhui Medical University, Hefei, Anhui Province, China. [3]Department of Anesthesiology, the Second Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Province 530007, China. [4]These authors contributed equally: Jiawei Zhang, Xiaoting Zheng.
✉email: liuxuesheng@ahmu.edu.cn; yangzhilai@163.com

Received: 10 April 2024 Revised: 8 May 2025 Accepted: 2 June 2025
Published online: 17 June 2025

with chronic pain is higher than in the general population, and patients with PTSD often experience chronic pain [18]. Clinical studies found that 3.5 to 4.7% of people experienced PTSD in the USA; however, the rate of PTSD was much higher in the chronic pain population (9 to 10%) [18]. Numerous clinical studies have found that chronic pain is comorbid with PTSD, but the mechanisms underlying this phenomenon are still unclear due to the lack of animal studies. Postoperative persistent pain, a type of chronic pain, is common and harmful in clinical practice. The SMIR model is a commonly used animal model for postoperative persistent pain [19, 20]. In this study, we used the SMIR model in mice to investigate the molecular mechanisms underlying the comorbidity between chronic pain and PTSD.

NMDARs are seven-transmembrane proteins, composed of four subunits, including NR1 and NR2A/NR2B. NMDARs are widely expressed in the nervous system and play crucial roles in synaptic plasticity, long-term potentiation (LTP), neuronal survival and death, and neurological diseases [21–23]. Activated NMDARs open channels to allow Ca^{2+} influx selectively activating downstream signaling pathways to produce biological functions [24]. Numerous studies have shown that NMDARs-related signaling pathways are important for pain perception [25], but their role in pain-related comorbidities requires further study. Rodent studies have found that injecting the NMDA receptor antagonist MK-801 into the ventral hippocampus of healthy mice impairs consolidation after fear extinction learning [26]. Additionally, the NMDA receptor partial agonist D-cycloserine (DCS) promotes fear extinction and reduces fear recovery [27–30]. Similarly, NMDA receptor activation has been shown to promote fear extinction in human studies [31]. Consequently, some researchers have proposed treating PTSD by activating NMDARs [21]. However, whether the contextual fear extinction dysfunction in the SMIR model is caused by NMDARs requires further exploration.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophic factor family, is widely expressed in the hippocampus and participates in neuroplasticity by activating tyrosine kinase receptor B (TrkB) in the postsynaptic density. Many studies have shown that the BDNF-related signaling pathway is vital for pain [32], but its role in pain-related comorbidities remains unclear. Additionally, BDNF plays a key role in fear extinction [33]. Studies have found that increasing BDNF protein content in the hippocampus can enhance contextual fear extinction learning [34]. Reduced BDNF expression, although not affecting the learning of contextual fear memory, altered hippocampus-dependent learning and retrieval of contextual fear extinction [33]. Additionally, reduced BDNF expression interfered with BDNF/TrkB signal transduction and induced dendritic spine loss [35]. Some studies have indicated that the BDNF/TrkB signaling pathway is indispensable for the generation and maturation of dendritic spines [36]. Postsynaptic density protein 95 (PSD-95) is the major scaffolding protein of glutamatergic synapses and interacts with NMDARs to regulate synaptic plasticity [37]. Overexpression of BDNF and TrkB can increase PSD-95 expression and enhance synaptic plasticity [38]. Synaptic plasticity also plays an important role in fear extinction [21]. Activating NMDA receptors can upregulate BDNF expression and enhance synaptic activity, thereby promoting contextual fear extinction [9]. However, the functions of the BDNF/TrkB signaling pathway in fear extinction in SMIR model mice are not clear.

Extracellular signal-regulated kinase (ERK), a downstream effector of NMDARs, can enhance cAMP response element-binding protein (CREB) activity, thereby promoting synaptic plasticity and BDNF synthesis [39, 40]. Upon activation of NMDA receptors, ERK phosphorylation increases [9], which regulates protein expression in the hippocampus under fear conditioning and enhances extinction learning [41]. Therefore, BDNF expression may be regulated through the NMDARs/ERK/CREB signaling pathway.

Overall, we hypothesize that chronic postoperative pain causes fear extinction dysfunction by inhibiting the NMDARs/ERK/CREB/BDNF signaling pathway in the hippocampus. NMDARs and BDNF/TrkB signaling pathways play crucial roles in contextual fear extinction and might serve as target molecules for PTSD. Many clinical studies have revealed the comorbidity between chronic pain and PTSD, but the molecular mechanisms remain poorly understood. We hope that our study may partially explain the molecular mechanisms underlying this comorbidity.

MATERIALS AND METHODS

Animals

Eight-week-old C57BL/6 male mice (25–30 g) were purchased from Jiangsu Jicui Yaokang Biotechnology Co., Ltd., with certificates of qualification for all mice. All animal operations in this study strictly followed relevant regulations and guidelines and were approved by the Animal Care Committee of Anhui Medical University. The animals were kept under standard conditions (12-h light/dark cycle, temperature 22 ± 1 °C, relative humidity 50% ± 10%) with adequate food and water. All efforts were made to minimize the number and suffering of experimental animals.

Surgery and drug injections

Following previous studies [19, 42], we imitated and modified the surgery procedure. The mice were placed in the supine position under 3% sevoflurane anesthesia, and the skin of the right inner thigh was prepared. The shaved skin is then scrubbed repeatedly with a sterile alcohol swab to disinfect the area and expose the great saphenous vein. A 1.0–1.3 cm incision was made on the inner thigh skin about 3 mm medial to the great saphenous vein to expose the thigh muscles. An incision about 1 cm long was then made in the superficial thigh (gracilis) muscle layer, about 3 mm medial to the saphenous nerve. By spreading blunt scissors over the muscle incision, the superficial muscle was further separated to allow insertion of a custom dissecting retractor. The skin and superficial thigh muscles were then retracted by 1 cm to expose the inferior adductor fascia, and the stretching continues for 1 h. Mice in the Sham group received the same anesthesia and surgery, but no skin or muscle stretching was performed. After recovery from anesthesia, all animals were able to walk normally and had normal access to food and water.

The mice were placed in a stereotaxic instrument, and guide cannulae (26 gauge; Plastics One, RWD Life Science) were implanted in 1.4 mm above the dorsal side of the bilateral hippocampus, using CA1 coordinates: −1.18 mm AP; +2.18 mm ML; −1.9 mm DV (Fig. S1). The cannula was fixed on the skull with dental cement, and a stainless steel core was inserted into each casing to keep it unobstructed and prevent infection. The mice were allowed to recover for at least one week after operation.

The NMDARs agonist NMDA (N-methyl-D aspartic acid) and the TrkB receptor inhibitor ANA-12 were both purchased from Sigma-Aldrich (USA) and dissolved in 0.9% normal saline. Following previous studies, each mouse was bilaterally co-infused with 0.005 μg of NMDA [43] and ANA-12 at a concentration of 10 μM [9]. To avoid damage to the hippocampus due to drug injection, ANA-12 was injected immediately after NMDA. Each side of the hippocampus was injected with 1 μl at a rate of 0.06 μl/min, followed by a 2-min diffusion time. The animals were returned to their original cages after the intracranial injection of drugs.

Behavioral experiments

Contextual fear conditioning and extinction. As mentioned earlier [44], the behavioral environments were conducted in a conditioned reflex chamber (Harvard Apparatus, Holliston, MA, USA). The mice were habituated by gentle handling for 5 min per day for three days before fear conditioned. On the first day, the mice adapted to the behavioral environment for 3 min and then received three foot shocks (0.60 mA, 2 s) at 180 s, 250 s, and 320 s. After the third shock, they remained in the chamber for 80 s before being returned to their cage. Twenty-four hours after fear conditioning, the mice underwent 45 min of extinction training without foot shock in the same chamber. After training, the mice were returned to their original cages for 24 h. On the third day, the mice were placed in the same chamber for a 5-min extinction memory retrieval experiment. During the training and testing, the movement of the mice on the grid floor was recorded by the Panlab Startle and Fear combined system with Packwin 2.0 software (Harvard Apparatus, Holliston, MA, USA) as shock amplitude. The degree of fear was assessed by freezing behavior, defined as complete immobility

except for breathing, and was recorded using Panlab Startle and Fear combined system with Packwin 2.0 software (Freeze Frame Actimetrics software).

Mechanical allodynia test. The mechanical pain threshold was measured at various time points after establishing the mouse model [42]. The electronic Von Frey apparatus (IITC Life Science, US) was used to measure the withdrawal threshold of the foot under mechanical stimulation. The force causing the withdrawal reflex of each right hind paw was recorded and averaged.

Animal experiment protocol

Mice were randomly divided into the following six groups: Control、Sham、SMIR、SMIR+Vehicle、SMIR + NMDA、SMIR + ANA-12. After SMIR or Sham operation, mechanical pain at the bottom of the right hindfoot was measured using an electronic Von Frey apparatus on the 1st, 3rd, 5th, 7th, 10th, 14th, 21st and 28th days. Contextual fear conditioned, extinction and retrieval experiments were conducted on the 8th-10th days. The SMIR operation was performed after intracranial catheterization in the mice. Contextual fear conditioning was conducted on the 8th day after the operation. After the panic state of the mice subsided, Vehicle or NMDA or ANA-12 was injected into the brain. Extinction training and retrieval experiments were carried out on the 9th and 10th days. After the behavioral tests, the mice were euthanized, and brain tissue was collected for the follow-up experiments.

Western blot analysis

Hippocampal tissue was collected on day 9 post-surgery. The samples were lysed to extract proteins as described previously [45, 46]. The sample were heated at 95 °C for 15 min to denature the proteins. Proteins in the lysed tissue were separated using 10% and 13.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (PVDF) (Millipore, Bedford, MA, USA). The membrane was blocked using TBST containing 0.1% Tween-20 and 5% skim milk, followed by incubation with the following antibodies: anti-NR2A(1:1000, #4205S, Cell Signaling Technology, USA); anti-NR2B(1:1000, #14544S, Cell Signaling Technology, USA); anti-ERK1/2(1:1000, #4695 T, Cell Signaling Technology, USA); anti-P-p44/42MAPK(1:1000, #9101S, Cell Signaling Technology, USA); anti-CREB(1:1000, #9197 T, Cell Signaling Technology, USA); anti-P-CREB(1:1000, #9198S, Cell Signaling Technology, USA); anti-BDNF(1:1000, #EPR1292, Abcam, USA); anti-β3-Tubllin(1:1000, #5666S, Cell Signaling Technology, USA); anti-PSD-95(1:1000, #2507S, Cell Signaling Technology, USA); anti-TrkB(1:1000, #4603, Cell Signaling Technology, USA); anti-TrkB(B-3) (1:500, #sc-7268, Santa Cruz Biotechnology, USA). The membranes were then incubated with HRP-conjugated anti-rabbit and anti-mouse IgG (Cell Signaling Technology, USA) for 1 h at room temperature and washed. The membranes were then incubated with chemiluminescence detection reagents (ECL, BL520A, Bioshap, Hefei, China) and visualized using a chemiluminescence apparatus (ImageQuant LAS 4000, GE Healthcare, Pittsburgh, PA, USA). Protein content was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

RNA extraction and real-time qPCR

The mRNA from hippocampal tissue was extracted using the HiPure Total RNA Mini Kit (Magen Biotech Co., Ltd). Single-stranded cDNA was synthesized using a reverse transcription kit (#RR036A, TakaRa). The expression of mRNA was detected by DNA amplification kit (#RR820A, TakaRa) and fast real-time qPCR system. The following primers are used: BDNF-R GAGCATCACCCGGGAAGTGT; BDNF-F CAAGGCAACTTGGCCTACCC; β-actin-R CACGATGGAGGGGAATACAG; β-actin-F CAGCTTCTTTG-CAGCTCCTT. The mRNA levels of BDNF and β-actin were normalized to β-actin as an internal control.

Golgi-cox

After extraction, the mouse brain tissue was immediately placed in fixative (Servicebio, G1101) and fixed at room temperature for over 48 h. The brain tissue was cut into 2–3 mm thick sections, rinsed gently several times with normal saline, then completely immersed in Golgi-Cox staining solution (Servicebio, G1069), and placed in a cool, ventilated, and light-avoided area for 14 days. The staining solution was changed after the initial 48 h and then every 3 days for a total of 14 days. Subsequently, the tissue was washed three times with distilled water and placed in 80% glacial acetic acid solution overnight. After softening, the tissue was washed with distilled water and placed in 30% sucrose solution. The tissue was cut into 100 μm thick sections using an oscillating microtome, attached to gelatin slides, and dried overnight in the dark. The tissue slides were then treated with concentrated ammonia for 15 min, washed with distilled water for 1 min, treated with acidic film fixative for 15 min, washed with distilled water for 3 min, dried, and sealed with glycerin gelatin (Servicebio, G1402). A digital slide scanner was used to perform panoramic multi-slice scanning to obtain a comprehensive image of the brain tissue. Image acquisition and analysis were performed using a microscope.

Transcriptome sequencing

Five mice were randomly selected from each of the Sham and SMIR groups, and hippocampus samples were sent to OEBiotech (Shanghai, China) for RNA extraction and sequencing. The thresholds for significantly different genes (DEGs) were set at a corrected p-value of 0.05 and a log2(fold change) of 1.0. Differential gene expression was analyzed using KEGG and WikiPathways enrichment, followed by Gene Set Enrichment Analysis (GSEA).

Statistical analysis

All sample sizes were determined based on paradigms used in previous similar studies [44]. Statistical analyses were performed using GraphPad Prism version 9.5.1 and ImageJ. F-tests were used to confirm the mean square error of the data. Normally distributed data were expressed as mean ± SEM, with n representing the number of animals or samples. Each point represents one sample. Two-way repeated measures (RM) ANOVA followed by Tukey's multiple comparisons test was used to detect significant differences among multiple groups of behavioral data. Other data from multiple groups were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Significant was set at $P < 0.05$.

RESULTS

SMIR mice develop contextual fear extinction dysfunction

As a previous study reported that SMIR mice did not exhibit thermal allodynia [19], we only performed mechanical allodynia measurements in this study. The mechanical pain threshold in the right hind paw was measured using an electronic Von Frey device on days 1, 3, 5, 7, 10, 14, 21, and 28 after SMIR surgery (Fig. 1A). Compared with the Control group, SMIR mice exhibited obvious pain behaviors, while the Sham group did not. Mechanical allodynia in SMIR mice persisted from the first to the 28th postoperative day (Fig. 1B).

Mice underwent a 3-day contextual fear extinction protocol as previously described (Fig. 1A). The 8th and 21st post-surgery days were selected for the contextual fear conditioning (CFC) experiment. Compared with the Control group, the SMIR group did not exhibit abnormal fear memory learning (Fig. 1C, S4A). Similarly, in the fear memory retrieval experiment, the SMIR group exhibited similar fear memory to the Control group (Fig. S2). In our experiments, during the extinction learning process, the freezing behavior of control mice decreased rapidly and maintained at a low level. However, the SMIR group exhibited a clear fear extinction dysfunction, manifested as impairment in extinction learning and extinction memory retrieval (Fig. 1D, E, S4B, S4C). To exclude the influence of surgery and anesthesia on fear extinction, we compared the Control group with the Sham group and found that the Sham group did not exhibit fear extinction dysfunction (Fig. 1C, D, E). Additionally, we evaluated locomotor activity and found that the operation had no effect on the locomotor activity of mice (Fig. S5). We also evaluated spatial memory using the Y-maze and found that the operation had no effect on the spatial memory of mice (Fig. S6). The data indicate that SMIR mice exhibit obvious fear extinction dysfunction.

The BDNF/TrkB signaling pathway was inhibited in SMIR mice

To further understand the gene expression in the hippocampus of SMIR mice, we performed transcriptomic analysis. We identified 370 differentially expressed genes (DEGs) in hippocampal

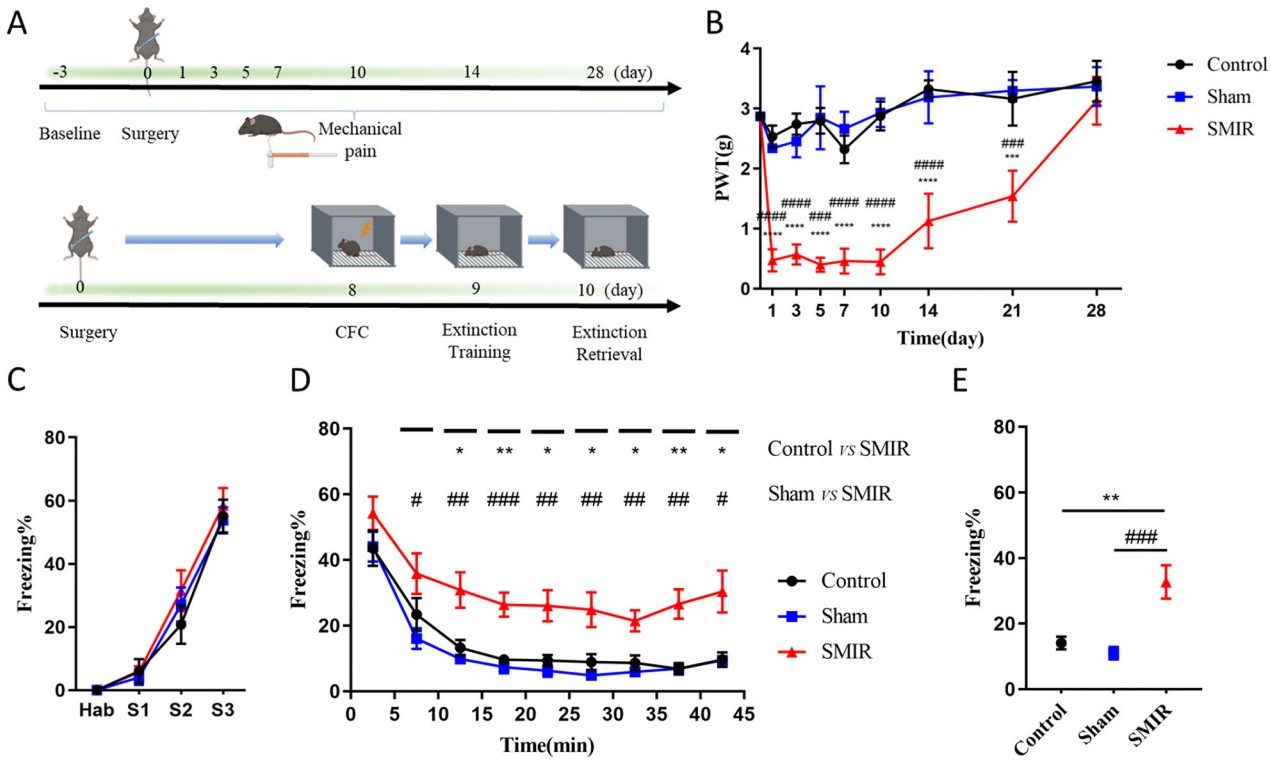

Fig. 1 SMIR Mice Develop Contextual Fear extinction dysfunction. A Experimental Flow Chart. **B** Mechanical allodynia was observed with PWT (n = 6). Differences between groups were adopted two-way RM (Repeated-measures) ANOVA followed by Tukey's multiple comparisons test. ***p < 0.001, ****p < 0.0001 vs Control; ###p < 0.001, ####p < 0.0001 vs Sham. **C** Contextual Fear Conditioning Experiment (n = 12). Differences between groups were adopted two-way RM (Repeated-measures) ANOVA followed by Tukey's multiple comparisons test. **D** Contextual Fear Extinction Experiment (n = 12). Differences between groups were adopted two-way RM (Repeated-measures) ANOVA followed by Tukey's multiple comparisons test. **E** Contextual Fear Retrieval Experiment (n = 12). Differences between groups were adopted one-way ANOVA followed by Tukey's multiple comparisons test. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

samples, including 255 up-regulated and 115 down-regulated genes (Fig. 2A, B). KEGG and WikiPathways enrichment analyses of the differential genes revealed potential signaling pathways, including Alzheimer's disease, the PI3K-AKT signaling pathway, the MAPK signaling pathway, and the cGMP-PKG signaling pathway (Fig. S3), which were partially consistent with published PTSD transcriptome results [47]. GSEA analysis further revealed the changes in the expression of genes related to BDNF signaling pathway, including the down-regulation of BDNF gene in SMIR mice (Fig. 2C, S3).

Based on the transcriptomic analysis results and the recognized importance of BDNF in fear extinction [33], we measured the expression of BDNF signaling pathway-related proteins, including BDNF and TrkB-full-length (TrkB-fl) proteins, on the 8th day post-surgery. Compared with the Control group and the Sham group, the expression of BDNF [F (2, 12) = 6.992, p < 0.05; Fig. 2D, E] and TrkB-fl [F (2, 9) = 9.245, p < 0.01; Fig. 2D, F] were reduced in the SMIR group, while there were no significant changes of BDNF and TrkB-fl between the Control group and the Sham group.

The NMDARs/ERK/CREB signaling pathway was inhibited in SMIR mice

As reported by previous studies, BDNF expression is regulated by the NMDARs/ERK/CREB signaling pathway [9]; therefore, we also measured the expression of NMDARs/ERK/CREB signaling pathway-related proteins. The hippocampus tissue of mice was removed on the 9th day post-surgery (Fig. 3H). Compared with the Control group and the Sham group, the expression of NR2B [F (2, 9) = 8.438, p < 0.05; Fig. 3A, B] and NR2A [F (2, 9) = 7.924, p < 0.05; Fig. 3A, C] were decreased in the SMIR group, while there were no significant changes of NR2B and NR2A between the Control group and the Sham group. Compared with the Control group and the Sham group, the expression of p-ERK/ERK [F (2, 9) = 12.98, p < 0.01; Fig. 3A, D] and p-CREB/CREB [F (2, 9) = 32.80, p < 0.001; Fig. 3A, E] were down-regulated in the SMIR group, while there were no significant changes of p-ERK/ERK and p-CREB/CREB between the Control group and the Sham group.

PSD-95 is a crucial scaffolding protein of the postsynaptic zone that interacts with NMDARs and plays a significant role in memory and synaptic plasticity [38]. Therefore, we examined the expression of PSD-95 proteins. Compared with the Control group and the Sham group, the expression of PSD-95 [F (2, 9) = 6.024, p < 0.05; Fig. 3F, G] was decreased in the SMIR group, while there was no significant change of PSD-95 between the Control group and the Sham group. Dendritic spines are also closely related to synaptic plasticity. Therefore, we examined the changes in dendritic spines in hippocampus. Compared with the Control group and Sham group, dendritic spines were missing in the SMIR group, while no significant difference was observed between the Control group and Sham group (Fig. 3I, J).

NMDARs agonist upregulates the expression of BDNF through NMDARs/ERK/CREB signaling pathway

To verify the upstream and downstream relationships among NMDARs, ERK/CREB, and BDNF, we injected NMDA [43], a specific agonist of NMDARs, into the hippocampus on the 8th day after surgery (Fig. 4H). It was confirmed that the mice did not exhibit epileptic behavior after drug injection, and hippocampal tissue samples were collected on the 9th day to detect protein expression. Compared with Sham group, the expressions of BDNF [F (3, 12) = 7.161, p < 0.05; Fig. 4A, B]、TrkB-fl [F (3, 12) = 9.103, p < 0.05; Fig. 4A, C]、p-ERK/ERK [F (3, 12) = 8.480, p < 0.05; Fig.

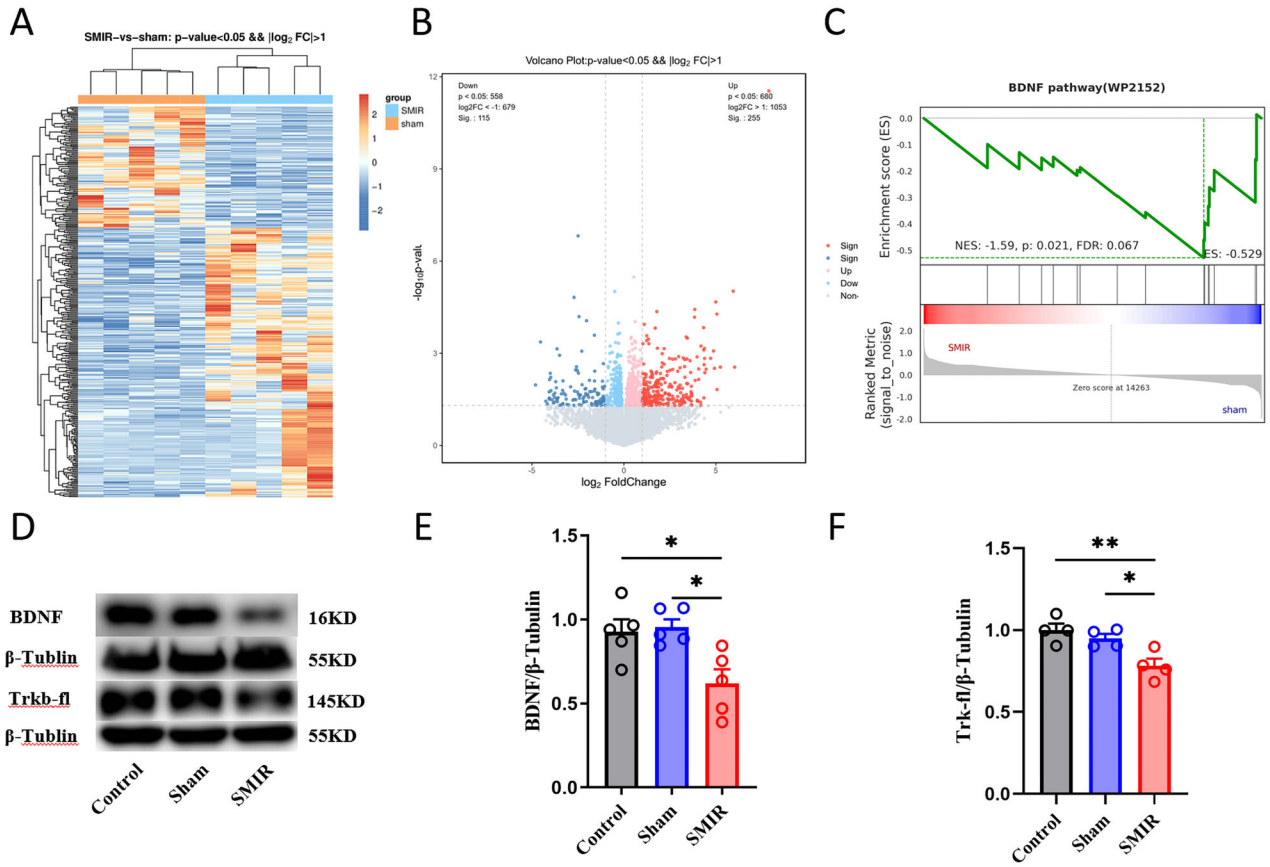

Fig. 2 Transcriptomic Analysis of Hippocampus in SMIR Mice. A The heatmap plot (Red represents relatively highly expressed protein-coding genes, and blue represents relatively low-expressed protein-coding genes) and **B** the volcano plot of differentially expressed genes in the hippocampus between the Sham and SMIR mice. **C** BDNF Pathway gene set enrichment analysis map (GSEA). **D** Representative Western blot analysis of BDNF and TrkB-fl (TrkB-full-length) in hippocampus. β-Tublin was included as loading Control. **E** Quantitative analysis of BDNF levels (n = 5). **F** Quantitative analysis of TrkB-fl (TrkB-full-length) levels (n = 4).

4D, E] and p-CREB/CREB [F (3, 12) = 522.8, p < 0.01; Fig. 4D, F] were down-regulated in the SMIR and SMIR+Vehicle groups. Compared with SMIR group, there were no differences in the expressions of BDNF [F (3, 12) = 7.161, p > 0.05; Fig. 4A, B]、TrkB-fl [F (3, 12) = 9.103, p > 0.05; Fig. 4A, C]、p-ERK/ERK [F (3, 12) = 8.480, p > 0.05; Fig. 4D, E] and p-CREB/CREB [F (3, 12) = 522.8, p > 0.05; Fig. 4D, F] in the SMIR+Vehicle group, while the expressions of BDNF [F (3, 12) = 7.161, p < 0.05; Fig. 4A, B]、TrkB-fl [F (3, 12) = 9.103, p < 0.05; Fig. 4A, C]、p-ERK/ERK [F (3, 12) = 8.480, p < 0.05; Fig. 4D, E] and P-CREB/CREB [F (3, 12) = 522.8, p < 0.0001; Fig. 4D, F] were up-regulated in the SMIR + NMDA group. Compared with the SMIR+Vehicle group, the expressions of BDNF [F (3, 12) = 7.161, p < 0.01; Fig. 4A, B]、TrkB-fl [F (3, 12) = 9.103, p < 0.01; Fig. 4A, C]、p-ERK/ERK [F (3, 12) = 8.480, p < 0.05; Fig. 4D, E] and p-CREB/CREB [F (3, 12) = 522.8, p < 0.0001; Fig. 4D, F] were up-regulated in the SMIR + NMDA group.

Meanwhile, we examined the mRNA expression levels of BDNF after NMDA administration to further validate the regulation of BDNF expression by NMDARs at the transcriptional level. Compared with the Sham group, mRNA [F (3, 12) = 8.144, p < 0.05; Fig. 4G] expression was reduced in both the SMIR and SMIR+Vehicle groups. There was no difference in mRNA [F (3, 12) = 8.144, p > 0.05; Fig. 4G] expression in the SMIR+Vehicle group compared with the SMIR group, whereas mRNA [F (3, 12) = 8.144, p < 0.05; Fig. 4G] expression was increased in the SMIR + NMDA group. Compared with the SMIR+Vehicle group, mRNA [F (3, 12) = 8.144, p < 0.05; Fig. 4G] expression was increased in the SMIR + NMDA group. These results indicated that activating NMDARs could activate the NMDARs/ERK/CREB signaling pathway, thereby upregulating the transcription of BDNF and increasing the expression level of BDNF protein.

NMDARs agonist improves contextual fear extinction dysfunction in SMIR mice by inducing PSD-95 expression and increasing dendritic spines in the hippocampus

Many studies have demonstrated that activating NMDARs can promote fear extinction. We performed CFC training on the 8th day post-SMIR surgery, followed by intracranial NMDA injection into the hippocampus, and subsequent extinction and retrieval experiments on the 9th and 10th days (Fig. 5A). Our results indicated that, compared with the Sham group, mice in the SMIR group and SMIR+Vehicle group exhibited impaired extinction learning and reduced retrieval of extinction memory (Fig. 5B, C, D). No significant difference was observed between the SMIR group and the SMIR+Vehicle group (Fig. 5B, C, D). Compared with the SMIR group and SMIR+Vehicle group, NMDA treatment enhanced extinction learning and increased the retrieval of extinction memory in SMIR mice (Fig. 5B, C, D), suggesting that the NMDARs agonist ameliorated contextual fear extinction dysfunction in SMIR mice.

Studies have demonstrated that BDNF can promote the expression of PSD-95 [38], therefore, we analyzed the protein expression of PSD-95 after NMDA treatment. We found that hippocampal PSD-95 [F (3, 16) = 9.958, p < 0.01; Fig. 5E, F] expression was reduced in the SMIR and SMIR+Vehicle groups compared to the Sham group. No significant difference in hippocampal PSD-95 [F (3, 16) = 9.958, p > 0.05; Fig. 5E, F] was observed between the SMIR group and the SMIR+Vehicle group.

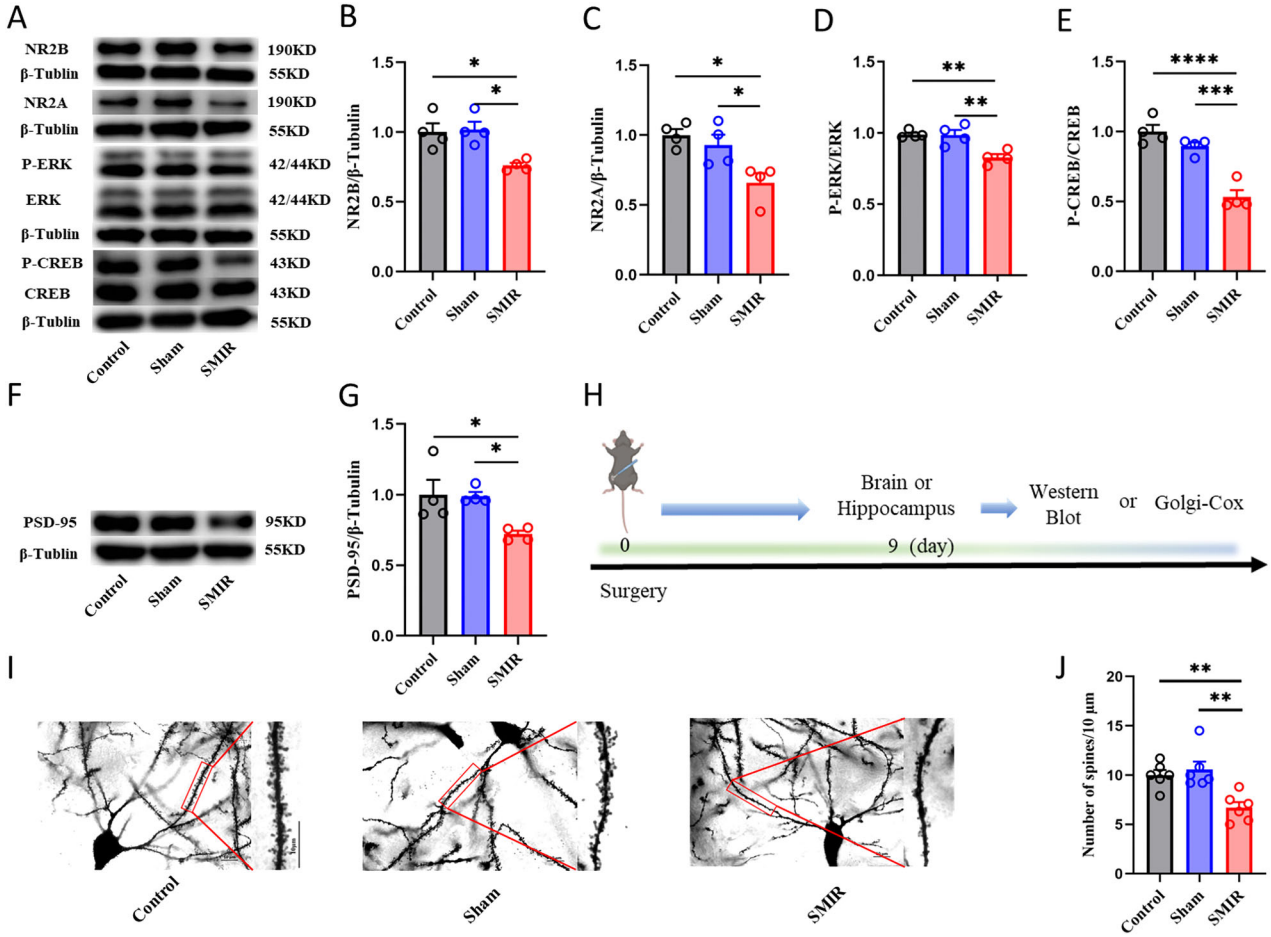

Fig. 3 The BDNF/TrkB and NMDARs/ERK/CREB Signaling Pathways were Inhibited in SMIR Mice. A Representative Western blot analysis of NR2B、NR2A、P-ERK、ERK、P-CREB and CREB in hippocampus. β-Tublin was included as loading control. **B** Quantitative analysis of NR2B levels (n = 4). **C** Quantitative analysis of NR2A levels (n = 4). **D** Quantitative analysis of P-Erk1/2 levels (n = 4). **E** Quantitative analysis of P-CREB levels (n = 4). **F** Representative Western blot analysis of PSD-95 in hippocampus. β-Tublin was included as loading Control. **G** Quantitative analysis of PSD-95 levels (n = 4). **H** Experimental flow chart. **I** Representative map of dendritic spine density of neurons in hippocampus. **J** Quantitative statistics of dendritic spine density (n = 6). Data are presented as the mean ± SEM. *p < 0.05, **p < 0.01.

Hippocampal PSD-95 [F (3, 16) = 9.958, p < 0.05; Fig. 5E, F] expression was elevated in the SMIR + NMDA group compared to the SMIR and SMIR+Vehicle groups. These results indicated that activation of NMDARs could upregulate PSD-95 expression.

Studies have found that downregulation of BDNF expression can induce dendritic spine deletion [35], therefore, we examined the dendritic spines in the hippocampus. Compared with the Sham group, the SMIR and SMIR+Vehicle groups exhibited dendritic spines deletion (Fig. 5G, H). There was no significant difference in dendritic spine deletion between the SMIR group and the SMIR+Vehicle group (Fig. 5G, H). Dendritic spine density increased in the SMIR + NMDA group compared to the SMIR and SMIR+Vehicle groups. This suggested that SMIR surgery induced dendritic spine deletion in the mouse hippocampus (Fig. 5G, H), and activation of hippocampal NMDARs restored the deleted dendritic spines in SMIR mice.

TrkB receptor inhibitor (ANA-12) reverses NMDA-ameliorated contextual fear extinction dysfunction in SMIR mice by re-inducing hippocampal dendritic spine loss

To further confirm the upstream-downstream relationship between NMDARs and the BDNF signaling pathway, we administered ANA-12 immediately following intracranial injection of NMDA in SMIR mice (Fig. 6A). We found that mice in the Sham group, SMIR + NMDA group, and SMIR + NMDA + ANA-12 group all exhibited normal extinction learning, but mice in the SMIR + NMDA + ANA-12 group and SMIR group showed increased freezing time during memory retrieval (Fig. 6B–D). The results indicated that simultaneous treatment with NMDA and ANA-12 did not impair the process of extinction learning, but impaired the retrieval of extinction memory. Activating NMDARs can promote fear extinction, but the consolidation of extinction memory also requires the transduction of the BDNF/TrkB signaling pathway.

Consistent with previous studies [35], the loss of dendritic spines was induced by inhibiting the BDNF/TrkB signaling pathway. Our results indicated that the number of dendritic spines was similar in the Sham and SMIR + NMDA groups; however, the SMIR + NMDA + ANA-12 and SMIR groups exhibited dendritic spine loss (Fig. 6E, F), suggesting that ANA-12 reversed NMDA-ameliorated contextual fear extinction dysfunction in SMIR mice by re-inducing hippocampal dendritic spine loss.

DISCUSSION

Multiple clinical studies have found that chronic pain is comorbid with PTSD; however, the molecular mechanisms of this comorbidity remain unclear due to a lack of experimental animal studies. In the present study, we observed that SMIR mice exhibited impaired contextual fear extinction. Additionally, SMIR operation might inhibit the NMDARs/ERK/CREB signaling pathway in the hippocampus,

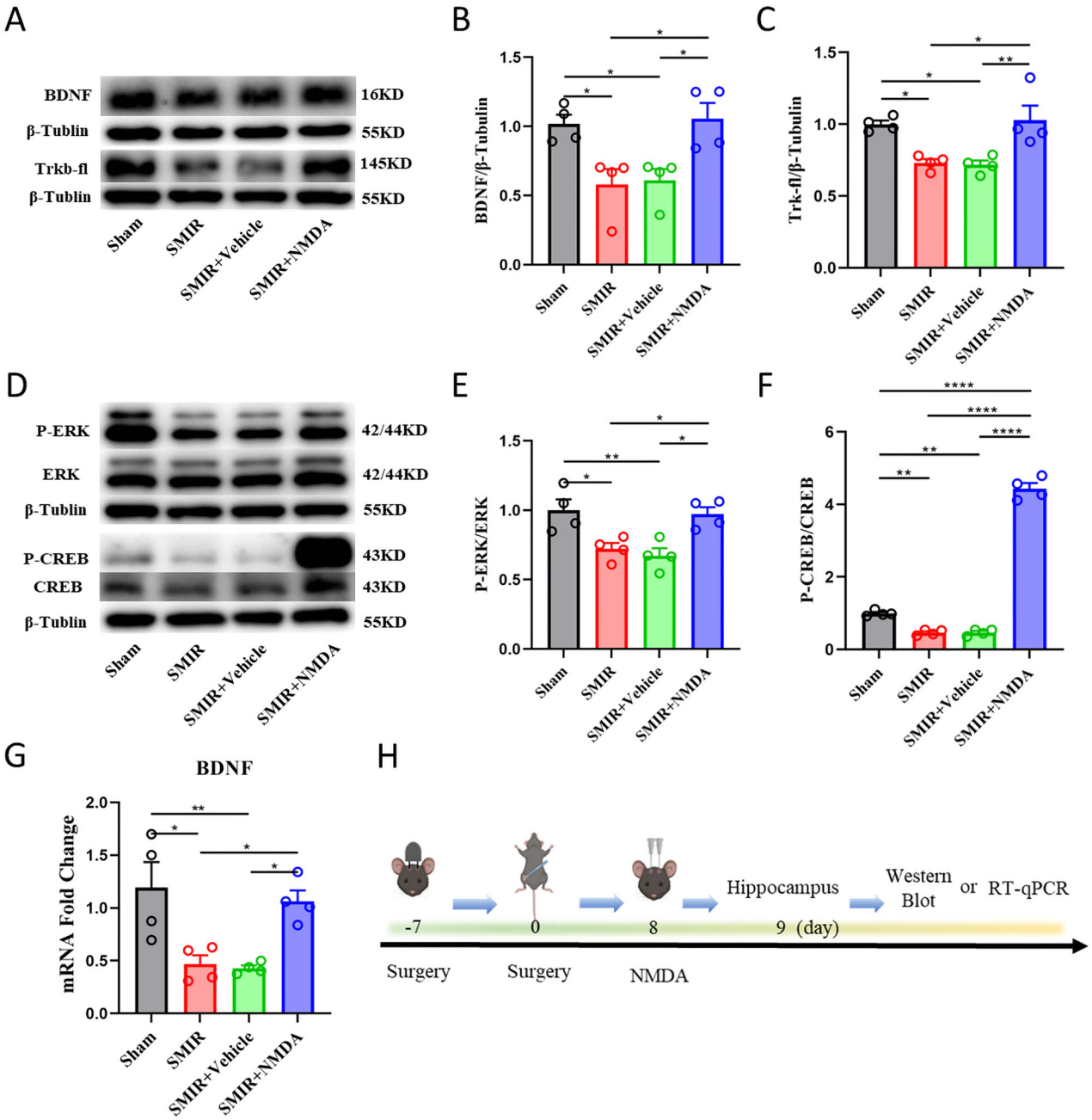

Fig. 4 NMDARs Agonist Upregulates the Expression of BDNF through NMDARs/ERK/CREB Signaling Pathway. A Representative Western blot analysis of hippocampal BDNF and TrkB-fl (TrkB-full-length) after NMDA treatment. β-Tubulin was included as a loading Control. **B** Quantitative analysis of BDNF levels (n = 4). **C** Quantitative analysis of TrkB-fl (TrkB-full-length) levels (n = 4). **D** Representative Western blot analysis of hippocampal P-Erk1/2 and P-CREB after NMDA treatment. Erk1/2 and CREB were included as loading Control. **E** Quantitative analysis of P-Erk1/2 levels (n = 4). **F** Quantitative analysis of P-CREB levels (n = 4). **G** Quantitative analysis of mRNA levels of hippocampus BDNF gene after NMDA treatment (n = 4). **H** Experimental flow chart. Data are presented as the mean ± SEM. *p < 0.05, **p < 0.01, ****p < 0.0001.

leading to the down-regulation of the BDNF/TrkB signaling pathway, thereby reducing PSD-95 expression and inducing dendritic spine loss in the hippocampus. Notably, activation of NMDARs promotes extinction learning in SMIR mice; however, the consolidation of extinction memory also requires the activation of the BDNF/TrkB signaling pathway. The comorbidity between chronic pain and PTSD is a common challenge in clinical practice. This study may partially elucidate the molecular mechanisms underlying the comorbidity between chronic pain and PTSD, and also validates the crucial role of NMDARs and the BDNF/TrkB signaling pathway in treating fear extinction dysfunctions in SMIR mice (Fig. 7).

Nowadays, the molecular mechanism underlying the comorbidity between chronic pain and PTSD is still unclear, and specific drugs for the treatment of patients with both chronic pain and PTSD are still lacking [11, 15, 16]. The incidence of PTSD in patients with chronic pain is higher than that in the general population [18]. One explanation is that chronic pain may induce PTSD-like symptoms in patients. Fear extinction dysfunction is considered one of the core features of PTSD, suggesting that chronic pain may affect fear extinction. A recent clinical study demonstrated that patients with chronic back pain (CBP) exhibited impaired extinction memory retrieval compared to healthy controls [48].

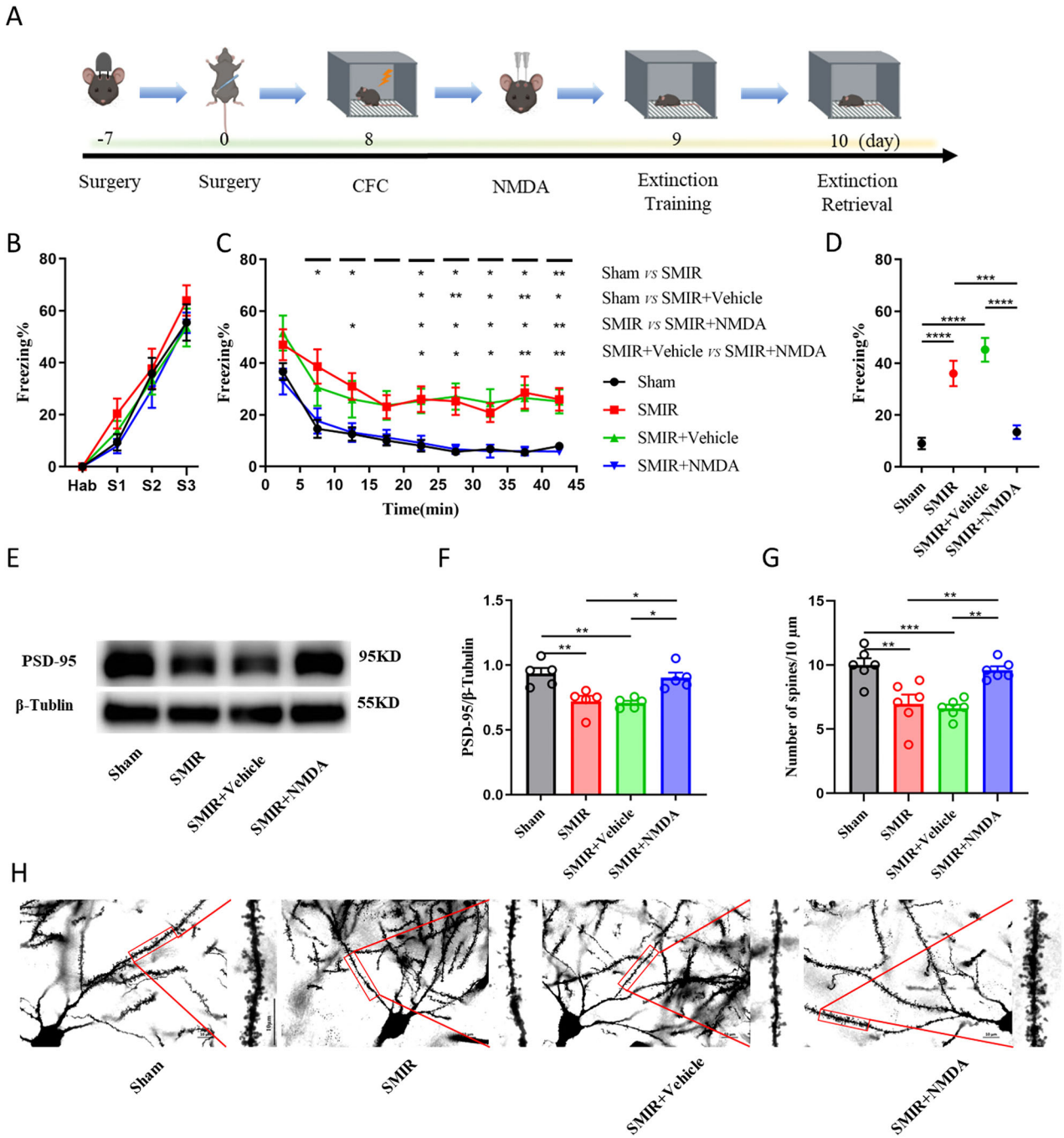

Fig. 5 NMDARs Agonist Improves Contextual Fear extinction dysfunction in SMIR Mice by Inducing PSD-95 Expression and Increasing Dendritic Spines in the Hippocampus. A Experimental flow chart. **B** Contextual Fear Conditioning Experiment (n = 11). Differences between groups were adopted two-way RM (Repeated-measures) ANOVA followed by Tukey's multiple comparisons test. **C** Contextual Fear Extinction Experiment (n = 11). Differences between groups were adopted two-way RM (Repeated-measures) ANOVA followed by Tukey's multiple comparisons test. **D** Contextual Fear Retrieval Experiment (n = 11). Differences between groups were adopted one-way ANOVA followed by Tukey's multiple comparisons test. **E** Representative Western blot analysis of hippocampal PSD-95 after NMDA treatment. β-Tubulin was included as a loading Control. **F** Quantitative analysis of PSD-95 levels (n = 5). **G** Quantitative statistics of dendritic spine density (n = 6). **H** Representative map of dendritic spine density of neurons in hippocampus. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

These clinical studies suggest that chronic pain can induce fear extinction dysfunction; however, there is currently no research on the mechanism through which chronic pain induces this disorder. According to previous study [19], chronic pain in mice in the current research was induced using a modified SMIR operation method. The SMIR mice exhibited hippocampus-dependent contextual fear extinction dysfunction, manifested by impaired extinction learning and memory retrieval. Our findings are consistent with the aforementioned clinical studies, where chronic pain induced by SMIR surgery led to fear extinction dysfunction in mice.

Many signaling pathways are implicated in the induction and maintenance of PTSD. Wang et al. conducted transcriptomic analysis on the hippocampus of PTSD mice induced by the SPS&S model and

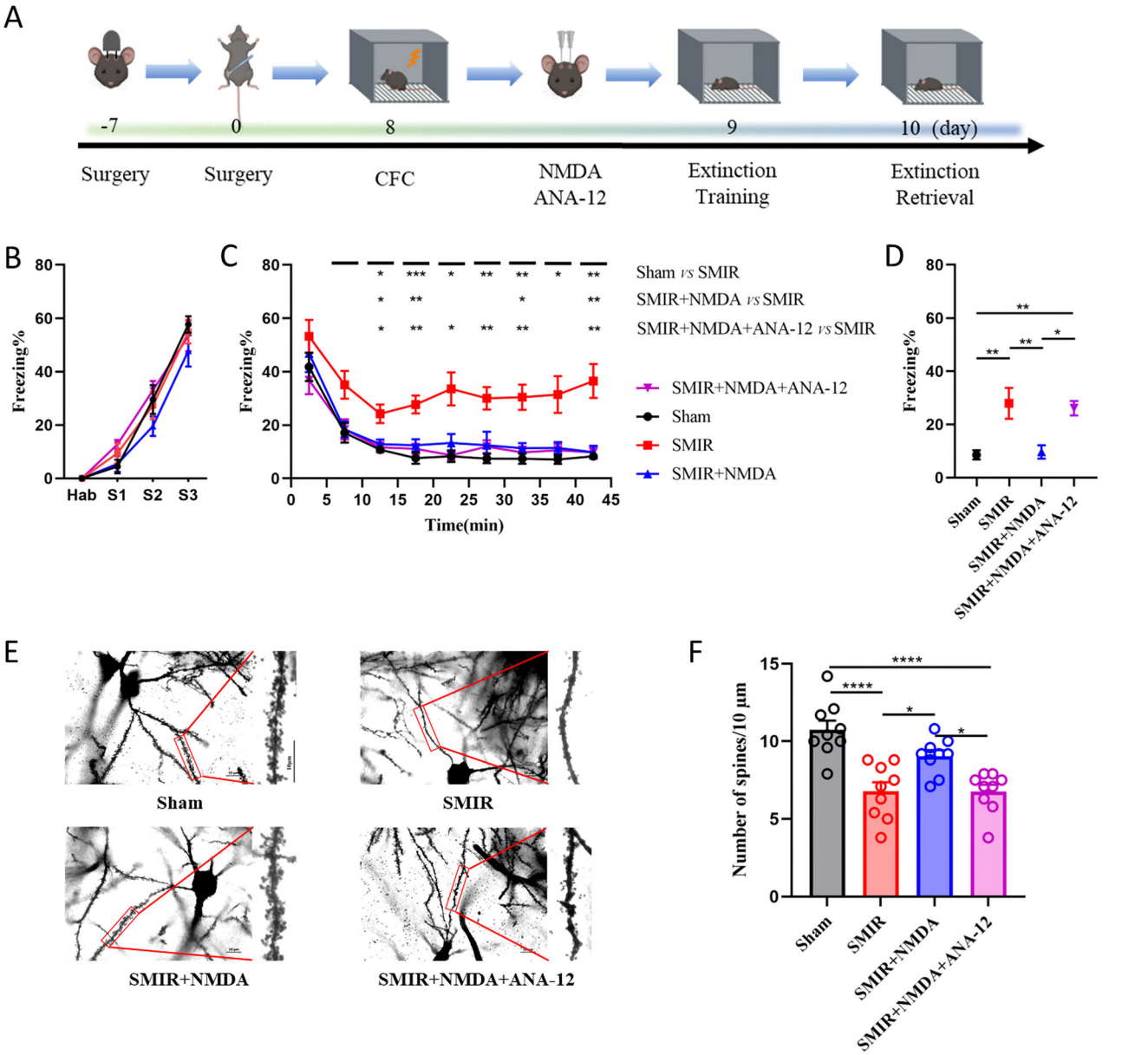

Fig. 6 TrkB Receptor Inhibitor (ANA-12) Reverses NMDA-ameliorated Contextual Fear extinction dysfunction in SMIR Mice by Inducing Hippocampal Dendritic Spine Loss. A Experimental flow chart. **B** Contextual Fear Conditioning Experiment (n = 11). Differences between groups were adopted two-way RM (Repeated-measures) ANOVA followed by Tukey's multiple comparisons test. **C** Contextual Fear Extinction Experiment (n = 11). Differences between groups were adopted two-way RM (Repeated-measures) ANOVA followed by Tukey's multiple comparisons test. **D** Contextual Fear Retrieval Experiment (n = 11). Differences between groups were adopted one-way ANOVA followed by Tukey's multiple comparisons test. **E** Representative map of dendritic spine density of neurons in hippocampus. **F** Quantitative statistics of dendritic spine density (n = 9). Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

found that differentially expressed genes (DEGs) were involved in the regulation of genes associated with Alzheimer's disease, mTOR signaling, long-term potentiation, PI3K-AKT signaling, and other pathways [47]. To investigate the molecular mechanisms of fear extinction dysfunction in SMIR mice, we also conducted transcriptomic analysis. Similar to the findings of Wang et al., DEGs were also involved in the regulation of genes related to Alzheimer's disease and the PI3K-AKT signaling pathway. Through gene set enrichment analysis (GSEA), we identified the down-regulation of genes related to BDNF signaling pathways in SMIR mice (Fig. 2). Considering the important role of the BDNF signaling pathway in fear extinction, we further examined the transduction and expression of BDNF signaling pathway-related genes and found down-regulation of this pathway in SMIR mice (Fig. 2).

As one of the most widely distributed neurotrophins in the mammalian brain, BDNF plays a critical role in development, neuronal survival, and particularly activity-dependent neuronal plasticity. Clinical studies have shown that humans with BDNF Val66Met alleles exhibit extinction learning disorders [33, 49]. In animal studies, $BDNF^{Met/Met}$ mice also exhibited fear extinction dysfunction, and after administration of exogenous BDNF, $BDNF^{Met/Met}$ mice showed restored extinction learning [50]. Generally, when the function or expression of BDNF was impaired, fear extinction was facilitated by administering exogenous BDNF to the brain, suggesting that BDNF is crucial for extinction learning [34, 51, 52]. Our study also found that the transcription and translation levels of BDNF were decreased in the hippocampus of SMIR mice. Moreover, the expression level of TrkB was also

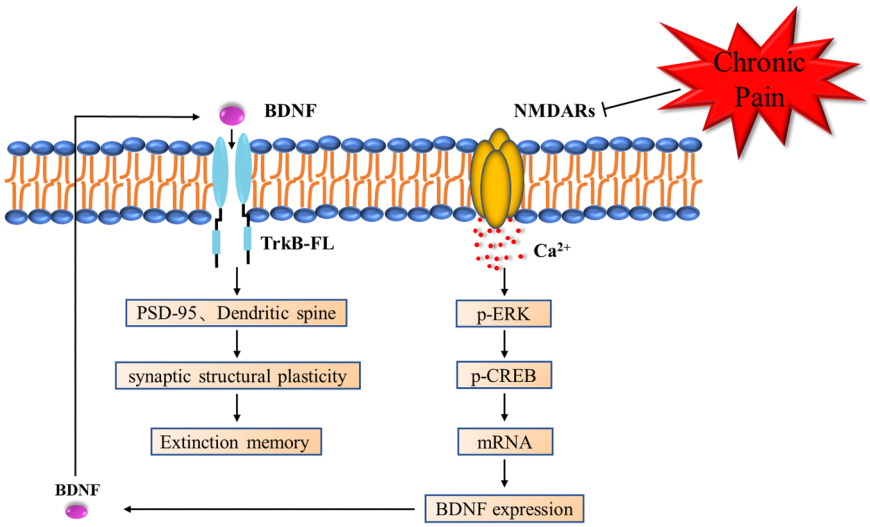

Fig. 7 Chronic pain causes inhibition of the NMDARs/ERK/CREB signaling pathway, resulting in inhibition of the BDNF/TrkB signaling pathway, down-regulation of PSD-95 expression and reduction of synaptic activity, thereby inducing fear extinction dysfunction.

decreased, and SMIR mice exhibited fear extinction dysfunction. These results suggeste that BDNF/TrkB plays a crucial role in fear extinction.

Numerous studies have demonstrated the crucial role of NMDARs in fear extinction and proposed strategies to treat PTSD by activating NMDARs [21]. Our study demonstrated decreased expression of NR2A and NR2B in the hippocampus and contextual fear extinction impairment in SMIR mice, consistent with the view that inhibition of NMDARs causes fear extinction impairment [9, 26, 28]. However, studies have found that in a rat model of chronic visceral pain, the expression of hippocampal NR2B is increased [53], and in a mouse model of chronic neuropathic pain, the expression of hippocampal NR2A is also increased [54]. The paradoxical expression of NMDARs may result from differences in animal species or types of chronic pain. Subsequently, we used NMDA, a specific agonist of NMDARs, to activate NMDARs. We found that treatment with NMDA promoted fear extinction learning in SMIR mice, similar to the results of extinction training immediately following DCS treatment [55]. Previous studies reported that NMDARs agonist treatment after extinction learning could enhance the consolidation of extinction memory [56, 57]. We also found that SMIR mice exhibited enhanced extinction memory retrieval following NMDA treatment. These studies collectively verified that the strategy of treating PTSD by activating NMDARs was reliable.

Studies have shown that activating NMDARs can induce an increase in BDNF gene expression in hippocampal neurons [58, 59]. Consistent with these studies, we found that activation of NMDARs increased the transcription and translation levels of BDNF. Additionally, we found that the expression of TrkB receptors in the hippocampus of SMIR mice was down-regulated, and this expression was up-regulated after treatment with NMDA. Wang et al. found that activating NMDARs can increase BDNF/TrkB signaling transduction, thereby promoting fear extinction [38], and one report indicated that blocking TrkB can impair memory consolidation [60]. Therefore, we speculated that SMIR mice treated with NMDA promoted the consolidation of extinction memory through the BDNF/TrkB signaling pathway. To test this hypothesis, we simultaneously injected NMDA and the TrkB receptor antagonist ANA-12 into the hippocampus. The results showed that the process of NMDA-restored extinction learning was not blocked by ANA-12 in SMIR mice, but NMDA-restored consolidation of extinction memory was impaired by ANA-12 in SMIR mice. These results indicated that the consolidation of extinction memory requires BDNF/TrkB signal transduction, and the BDNF/TrkB signaling pathway is downstream of NMDARs.

Generally, synaptic NMDARs transmit information through the activation of the survival signaling protein ERK, which drives the transcription of cAMP response element binding protein (CREB), promoting the synthesis of BDNF [39, 40]. We found that the activation of the ERK/CREB signaling pathway in the hippocampus of SMIR mice was inhibited, which may explain the reduction in BDNF expression. Following NMDA treatment, the ERK/CREB signaling pathway was activated, consistent with previous studies. These results suggest that BDNF expression may be regulated by the NMDAR/ERK/CREB signaling pathway in SMIR mice.

The role of synaptic plasticity in fear extinction has garnered widespread attention [4, 21]. The postsynaptic density protein PSD-95 and dendritic spines are closely related to synaptic plasticity [61, 62]. We found that the expression of PSD-95 was reduced in the hippocampus of SMIR mice, suggesting the destruction of synaptic plasticity in SMIR mice. Studies have shown that activating NMDARs or increasing BDNF can promote PSD-95 expression and enhance synaptic plasticity [38, 63]. Additionally, we found that the expression of PSD-95 in the hippocampus of SMIR mice increased after treatment with NMDA. Giza et al.'s study indicated [59] that BDNFVal66Met, a mutant gene of BDNF, could lead to a decrease in the expression of mature BDNF, inducing the loss of dendritic spines and thus impaired fear extinction This is consistent with our experimental results. Missing dendritic spines were observed in the hippocampus of SMIR mice. Furthermore, activation of the BDNF/TrkB signaling pathway promoted an increase in dendritic spine density [36]. As predicted, we found that lost dendritic spines were restored after treatment with NMDA, and dendritic spines were lost again in the hippocampus of SMIR mice treated with both NMDA and ANA-12. From this, we conclude that in the hippocampus of SMIR mice, activating NMDARs promotes fear extinction by activating BDNF/TrkB signal transduction, increasing synaptic PSD-95, and promoting the increase in dendritic spine density.

Multiple brain areas are involved in the regulation of fear extinction, including the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and ventral hippocampus (vHPC) [64]. The CA1 region of the hippocampus, dominated by pyramidal cells, is the main information output region of the hippocampus [65]. It receives and processes information from various brain regions inside and outside the hippocampus [66], and outputs information to regions such as the entorhinal cortex (EC) [67]. Research has shown that the anterior cingulate cortex

(ACC) and insular cortex (IC) play important roles in brain networks involved in pain and emotion [68, 69]. A clinical study using MRI showed that patients with PTSD had reduced volumes of the hippocampus and ACC [70]. Recent studies have found that neurocircuits from the ACC to the ventral hippocampus mediate contextual fear generalization [71], and there are also bidirectional neural projections between the ACC and the dorsal hippocampus [71]. However, whether the neural circuit between the ACC and hippocampus mediates fear extinction dysfunction remains unclear. It has also been suggested that the thalamic nucleus reuniens (NR)-CA1 pathway is necessary for normal fear extinction [66]. Our research also has many shortcomings. Although the hippocampus is a classic brain region for fear extinction dysfunction, it is not typically associated with chronic pain. However, our study shows significant molecular changes in the hippocampus of SMIR mice. A possible explanation is that pain-related brain areas, such as the anterior cingulate cortex, regulate the activity of the hippocampus. For example, the neurocircuits from the anterior cingulate cortex (ACC) to the ventral hippocampus, identified in previous studies, may be activated in chronic pain mice, based on the fact that the ACC is activated in chronic pain [10, 71, 72]. Additionally, the neurons in the hippocampus may be regulated by pain-related brain areas, potentially inducing molecular and plasticity changes in the hippocampus of chronic pain mice. The upstream brain areas of the hippocampus regulate fear extinction impairment still needs to be studied.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding authors on reasonable request.

REFERENCES

1. Liberzon I, Abelson JL. Context processing and the neurobiology of post-traumatic stress disorder. Neuron. 2016;92:14–30.
2. Kida S. Reconsolidation/destabilization, extinction and forgetting of fear memory as therapeutic targets for PTSD. Psychopharmacology. 2018;236:49–57.
3. Furini C, Myskiw J, Izquierdo I. The learning of fear extinction. Neurosci Biobehav Rev. 2014;47:670–83.
4. Luchkina NV, Bolshakov VY. Mechanisms of fear learning and extinction: synaptic plasticity-fear memory connection. Psychopharmacology (Berl). 2019;236:163–82.
5. Maren S, Phan KL, Liberzon I. The contextual brain: implications for fear conditioning, extinction and psychopathology. Nat Rev Neurosci. 2013;14:417–28.
6. Lacagnina AF, Brockway ET, Crovetti CR, Shue F, McCarty MJ, Sattler KP, et al. Distinct hippocampal engrams control extinction and relapse of fear memory. Nat Neurosci. 2019;22:753–61.
7. Bazaz A, Ghanbari A, Vafaei AA, Khaleghian A, Rashidy-Pour A. Oxytocin in dorsal hippocampus facilitates auditory fear memory extinction in rats. Neuropharmacology. 2022;202:108844.
8. Wang ME, Yuan RK, Keinath AT, Ramos Alvarez MM, Muzzio IA. Extinction of learned fear induces hippocampal place cell remapping. J Neurosci. 2015;35:9122–36.
9. Cai CY, Chen C, Zhou Y, Han Z, Qin C, Cao B, et al. PSD-95-nNOS coupling regulates contextual fear extinction in the dorsal CA3. Sci Rep. 2018;8:12775.
10. Cullen PK, Gilman TL, Winiecki P, Riccio DC, Jasnow AM. Activity of the anterior cingulate cortex and ventral hippocampus underlie increases in contextual fear generalization. Neurobiol Learn Mem. 2015;124:19–27.
11. Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. Lancet. 2021;397:2082–97.
12. Cui M, Ji R, Song L, Wang X, Pan X, Han Y, et al. Neuronal and molecular mechanisms underlying chronic pain and depression comorbidity in the paraventricular thalamus. J Neurosci. 2024;44:e1752232024.
13. Wei N, Guo Z, Qiu M, Ye R, Shao X, Liang Y, et al. Astrocyte activation in the ACC contributes to comorbid anxiety in chronic inflammatory pain and involves in the excitation-inhibition imbalance. Mol Neurobiol. 2024;61:6934–49.
14. Jadhakhan F, Evans DW, Falla D. The role of post-trauma stress symptoms in the development of chronic musculoskeletal pain and disability: a systematic review. Eur J Pain. 2023;27:183–200.
15. Morasco BJ, Lovejoy TI, Lu M, Turk DC, Lewis L, Dobscha SK. The relationship between PTSD and chronic pain: mediating role of coping strategies and depression. Pain. 2013;154:609–16.
16. Chopin SM, Sheerin CM, Meyer BL. Yoga for warriors: an intervention for veterans with comorbid chronic pain and PTSD. Psychol Trauma. 2020;12:888–96.
17. Hadlandsmyth K, Driscoll MA, Johnson NL, Mares JG, Mengeling MA, Thomas EBK, et al. Veterans with chronic pain: Examining gender differences in pain type, overlap, and the impact of post-traumatic stress disorder. Eur J Pain. 2024;28:1311–9.
18. Kind S, Otis JD. The interaction between chronic pain and PTSD. Curr Pain Headache Rep. 2019;23:91.
19. Flatters SJ. Characterization of a model of persistent postoperative pain evoked by skin/muscle incision and retraction (SMIR). Pain. 2008;135:119–30.
20. Wei SN, Zhang H, Lu Y, Yu HJ, Ma T, Wang SN, et al. Microglial VDAC1 signaling modulates sleep deprivation-induced transition to chronic postsurgical pain. Sleep. 2023;46:zsad039.
21. Battaglia S, Di Fazio C, Vicario CM, Avenanti A. Neuropharmacological modulation of N-methyl-D-aspartate, noradrenaline and endocannabinoid receptors in fear extinction learning: synaptic transmission and plasticity. Int J Mol Sci. 2023;24:5926.
22. Dupuis JP, Nicole O, Groc L. NMDA receptor functions in health and disease: Old actor, new dimensions. Neuron. 2023;111:2312–28.
23. Ge Y, Wang YT. GluN2B-containing NMDARs in the mammalian brain: pharmacology, physiology, and pathology. Frontiers in Molecular Neuroscience. 2023;16:1190324.
24. Martin HGS, Wang YT. Blocking the deadly effects of the NMDA receptor in stroke. Cell. 2010;140:174–6.
25. Zhuo M. Plasticity of NMDA receptor NR2B subunit in memory and chronic pain. Mol Brain. 2009;2:4.
26. Zhang B, Li CY, Wang XS. The effect of hippocampal NMDA receptor blockade by MK-801 on cued fear extinction. Behav Brain Res. 2017;332:200–3.
27. Tang S, Graham BM. d-Cycloserine and estradiol enhance fear extinction in nulliparous but not primiparous female rats. Neurobiol Learn Mem. 2019;166:107088.
28. Graham BM, Scott E. Estradiol-induced enhancement of fear extinction in female rats: The role of NMDA receptor activation. Prog Neuropsychopharmacol Biol Psychiatry. 2018;86:1–9.
29. Ledgerwood L, Richardson R, Cranney J. D-cycloserine facilitates extinction of learned fear: effects on reacquisition and generalized extinction. Biol Psychiatry. 2005;57:841–7.
30. Ledgerwood L, Richardson R, Cranney J. D-cycloserine and the facilitation of extinction of conditioned fear: consequences for reinstatement. Behav Neurosci. 2004;118:505–13.
31. Inslicht SS, Niles AN, Metzler TJ, Lipshitz SL, Otte C, Milad MR, et al. Randomized controlled experimental study of hydrocortisone and D-cycloserine effects on fear extinction in PTSD. Neuropsychopharmacology. 2022;47:1945–52.
32. Cao T, Matyas JJ, Renn CL, Faden AI, Dorsey SG, Wu J. Function and mechanisms of truncated BDNF receptor TrkB.T1 in neuropathic pain. Cells. 2020;9:1194.
33. Notaras M, van den Buuse M. Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders. Mol Psychiatry. 2020;25:2251–74.
34. Liu IYC, Lyons WE, Mamounas LA, Thompson RF. Brain-Derived neurotrophic factor plays a critical role in contextual fear conditioning. J Neurosci. 2004;24:7958–63.
35. Giza JI, Kim J, Meyer HC, Anastasia A, Dincheva I, Zheng CI, et al. The BDNF Val66Met prodomain disassembles dendritic spines altering fear extinction circuitry and behavior. Neuron. 2018;99:163–178.e166.
36. Zagrebelsky M, Tacke C, Korte M. BDNF signaling during the lifetime of dendritic spines. Cell Tissue Res. 2020;382:185–99.
37. Steiner P, Higley MJ, Xu W, Czervionke BL, Malenka RC, Sabatini BL. Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. Neuron. 2008;60:788–802.
38. Wang J, Cai Y, Sun J, Feng H, Zhu X, Chen Q, et al. Administration of intramuscular AAV-BDNF and intranasal AAV-TrkB promotes neurological recovery via enhancing corticospinal synaptic connections in stroke rats. Exp Neurol. 2023;359:114236.
39. Pak ME, Li W. Neuroprotective effects of sparassis crispa ethanol extract through the AKT/NRF2 and ERK/CREB pathway in mouse hippocampal cells. Journal of Fungi. 2023;9:910.
40. Wang H, Peng RY. Basic roles of key molecules connected with NMDAR signaling pathway on regulating learning and memory and synaptic plasticity. Mil Med Res. 2016;3:26.
41. Glavonic E, Mitic M, Francija E, Petrovic Z, Adzic M. Sex-specific role of hippocampal NMDA-Erk-mTOR signaling in fear extinction of adolescent mice. Brain Res Bull. 2023;192:156–67.
42. Yang J, Yuan F, Ye G, Wang Y-J, Wu C, Wang J, et al. Skin/Muscle incision and retraction induces evoked and spontaneous pain in mice. Pain Res Manag. 2019;2019:1–9.
43. Rezayof A, Shirazi-Zand Z, Zarrindast MR, Nayer-Nouri T. Nicotine improves ethanol-induced memory impairment: the role of dorsal hippocampal NMDA receptors. Life Sci. 2010;86:260–6.

44. Zhang X, Kim J, Tonegawa S. Amygdala reward neurons form and store fear extinction memory. Neuron. 2020;105:1077–1093.e1077.
45. Qiu LL, Pan W, Luo D, Zhang GF, Zhou ZQ, Sun XY, et al. Dysregulation of BDNF/TrkB signaling mediated by NMDAR/Ca(2+)/calpain might contribute to post-operative cognitive dysfunction in aging mice. J Neuroinflammation. 2020;17:23.
46. Gu Z, Liu W, Yan Z. beta-Amyloid impairs AMPA receptor trafficking and function by reducing Ca2+/calmodulin-dependent protein kinase II synaptic distribution. J Biol Chem. 2009;284:10639–49.
47. Wang J, Gao F, Cui S, Yang S, Gao F, Wang X, Zhu G. Utility of 7,8-dihydroxyflavone in preventing astrocytic and synaptic deficits in the hippocampus elicited by PTSD. Pharmacol Res. 2022;176:106079.
48. Schmidt K, Schlitt F, Wiech K, Merz CJ, Kleine-Borgmann J, Wolf OT, et al. Hydrocortisone differentially affects reinstatement of pain-related responses in patients with chronic back pain and healthy volunteers. J Pain. 2023;25:1082–93.
49. Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, et al. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. Science. 2010;327:863–6.
50. Pattwell SS, Bath KG, Perez-Castro R, Lee FS, Chao MV, Ninan I. The BDNF Val66Met polymorphism impairs synaptic transmission and plasticity in the infralimbic medial prefrontal cortex. J Neurosci. 2012;32:2410–21.
51. Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. Induction of fear extinction with hippocampal-infralimbic BDNF. Science. 2010;328:1288–90.
52. Heldt SA, Stanek L, Chhatwal JP, Ressler K. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. Mol Psychiatry. 2007;12:656–70.
53. Chen Y, Chen AQ, Luo XQ, Guo LX, Tang Y, Bao CJ, et al. Hippocampal NR2B-containing NMDA receptors enhance long-term potentiation in rats with chronic visceral pain. Brain Res. 2014;1570:43–53.
54. Liu R, Sun D, Xing X, Chen Q, Lu B, Meng B, et al. Intranasal oxytocin alleviates comorbid depressive symptoms in neuropathic pain via elevating hippocampal BDNF production in both female and male mice. Neuropharmacology. 2024;242:109769.
55. Dubois CJ, Liu SJ. GluN2D NMDA receptors gate fear extinction learning and interneuron plasticity. Front Synaptic Neurosci. 2021;13:681068.
56. Baker KD, McNally GP, Richardson R. d-Cycloserine facilitates fear extinction in adolescent rats and differentially affects medial and lateral prefrontal cortex activation. Prog Neuropsychopharmacol Biol Psychiatry. 2018;86:262–9.
57. Shelkar GP, Liu J, Dravid SM. Astrocytic NMDA receptors in the basolateral amygdala contribute to facilitation of fear extinction. Int J Neuropsychopharmacol. 2021;24:907–19.
58. Tian F, Marini AM, Lipsky RH. NMDA receptor activation induces differential epigenetic modification of Bdnf promoters in hippocampal neurons. Amino Acids. 2010;38:1067–74.
59. Keyan D, Bryant RA. Acute exercise-induced enhancement of fear inhibition is moderated by BDNF Val66Met polymorphism. Transl Psychiatry. 2019;9:131.
60. Gross KS, Alf RL, Polzin TR, Frick KM. 17beta-estradiol activation of dorsal hippocampal TrkB is independent of increased mature BDNF expression and is required for enhanced memory consolidation in female mice. Psychoneuroendocrinology. 2021;125:105110.
61. Zeng M, Shang Y, Araki Y, Guo T, Huganir RL, Zhang M. Phase transition in postsynaptic densities underlies formation of synaptic complexes and synaptic plasticity. Cell. 2016;166:1163–1175.e1112.
62. Zanca RM, Sanay S, Avila JA, Rodriguez E, Shair HN, Serrano PA. Contextual fear memory modulates PSD95 phosphorylation, AMPAr subunits, PKMzeta and PI3K differentially between adult and juvenile rats. Neurobiol Stress. 2019;10:100139.
63. Yoshii A, Constantine-Paton M. BDNF induces transport of PSD-95 to dendrites through PI3K-AKT signaling after NMDA receptor activation. Nat Neurosci. 2007;10:702–11.
64. Liu Y, Ye S, Li XN, Li WG. Memory trace for fear extinction: fragile yet reinforceable. Neurosci Bull. 2023;40:777–94.
65. Geiller T, Priestley JB, Losonczy A. A local circuit-basis for spatial navigation and memory processes in hippocampal area CA1. Curr Opin Neurobiol. 2023;79:102701.
66. Ratigan HC, Krishnan S, Smith S, Sheffield MEJ. A thalamic-hippocampal CA1 signal for contextual fear memory suppression, extinction, and discrimination. Nat Commun. 2023;14:6758.
67. Roy DS, Kitamura T, Okuyama T, Ogawa SK, Sun C, Obata Y, et al. Distinct neural circuits for the formation and retrieval of episodic memories. Cell. 2017;170:1000–1012.e1019.
68. Lee JA, Chen Q, Zhuo M. Synaptic plasticity in the pain-related cingulate and insular cortex. Biomedicines. 2022;10:2745.
69. Barthas F, Sellmeijer J, Hugel S, Waltisperger E, Barrot M, Yalcin I. The anterior cingulate cortex is a critical hub for pain-induced depression. Biol Psychiatry. 2015;77:236–45.
70. O'Doherty DC, Chitty KM, Saddiqui S, Bennett MR, Lagopoulos J. A systematic review and meta-analysis of magnetic resonance imaging measurement of structural volumes in posttraumatic stress disorder. Psychiatry Res. 2015;232:1–33.
71. Bian XL, Qin C, Cai CY, Zhou Y, Tao Y, Lin YH, et al. Anterior cingulate cortex to ventral hippocampus circuit mediates contextual fear generalization. J Neurosci. 2019;39:5728–39.
72. Yu W, Wu X, Chen Y, Liang Z, Jiang J, Misrani A, et al. Pelvic pain alters functional connectivity between anterior cingulate cortex and hippocampus in both humans and a rat model. Front Syst Neurosci. 2021;15:642349.

ACKNOWLEDGEMENTS

We thank professor Er-wei Gu for proof readings and valuable comments.

AUTHOR CONTRIBUTIONS

Zhilia Yang and Xuesheng Liu are both corresponding authors and designed the research. Jiawei Zhang, Xiaoting Zheng, Gaoyan Zhang, Zhikun Cheng, Yinuo Liu, Lei Zhang and Jiqian Zhang performed the research and analyzed the data. All authors contributed to the writing and critical assessment of the manuscript. Jiawei Zhang and Xiaoting Zheng contributed equally to this study.

FUNDING

This study is supported by Scientific Research Project of Anhui Provincial Health Commission (No. AHWJ2023A20031), Fund Project of Anhui Medical University (No. 2023xkj150), National Natural Science Foundation of China (No. 81500949) and Excellent Young Talents Fund of Higher School in Anhui Province (No. gxyq2018009) to Zhilai Yang. National Natural Science Foundation of China (No. 82171192) to Xuesheng Liu.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal treatment procedures were conducted in compliance with international guidelines (NIH Publications No. 8023, revised in 1978) and the Chinese Council's Guides for the Care and Use of Laboratory Animals. The protocols in this study were approved by the Animal Care Committee of Anhui Medical University.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41398-025-03417-0.

Correspondence and requests for materials should be addressed to Xuesheng Liu or Zhilai Yang.