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Upregulation of Phosphodiesterase 7A Contributes to Concurrent Pain and Depression via Inhibition of cAMP-PKA-CREB-BDNF Signaling and Neuroinflammation in the Hippocampus of Mice

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Abstract

Background: Phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cyclic adenosine monophosphate AMP (cAMP) and/or cyclic guanosine monophosphate (cGMP). PDE inhibitors can mitigate chronic pain and depression when these disorders occur individually; however, there is limited understanding of their role in concurrent chronic pain and depression. We aimed to evaluate the mechanisms of action of PDE using 2 mouse models of concurrent chronic pain and depression.

Methods: C57BL/6J mice were subjected to partial sciatic nerve ligation (PSNL) to induce chronic neuropathic pain or injected with complete Freund's adjuvant (CFA) to induce inflammatory pain, and both animals showed depression-like behavior. First, we determined the change in PDE expression in both animal models. Next, we determined the effect of PDE7 inhibitor BRL50481 or hippocampal *PDE7A* knockdown on PSNL- or CFA-induced chronic pain and depression-like behavior. We also investigated the role of cAMP-protein kinase A (PKA)-cAMP response element binding protein (CREB)-brain-derived neurotrophic factor (BDNF) signaling and neuroinflammation in the effect of PDE7A inhibition on PSNL- or CFA-induced chronic pain and depression-like behavior.

Results: This induction of chronic pain and depression in the 2 animal models upregulated hippocampal PDE7A. Oral administration of PDE7 inhibitor, BRL50481, or hippocampal *PDE7A* knockdown significantly reduced mechanical hypersensitivity and depression-like behavior. Hippocampal PDE7 inhibition reversed PSNL- or CFA-induced downregulation of cAMP and BDNF and the phosphorylation of PKA, CREB, and p65. cAMP agonist forskolin reversed these changes and caused milder behavioral symptoms of pain and depression. BRL50481 reversed neuroinflammation in the hippocampus in PSNL mice.

Conclusions: Hippocampal PDE7A mediated concurrent chronic pain and depression in both mouse models by inhibiting cAMP-PKA-CREB-BDNF signaling. Inhibiting PDE7A or activating cAMP-PKA-CREB-BDNF signaling are potential strategies to treat concurrent chronic pain and depression.

Keywords: Chronic pain, depression, phosphodiesterase7A, cyclic adenosine monophosphate, neuroinflammation

Significance Statement

Inhibitors of phosphodiesterase (PDE) can mitigate chronic pain and depression when these disorders occur individually; however, there is limited understanding of their role and mechanism of action in concurrent chronic pain and depression. PDE7 hydrolyzes cyclic AMP in the central nervous system. This study aimed to clarify how PDE7A contributes to concurrent pain and depression and how its inhibition exerts therapeutic effects. This study revealed that PDE7A mediates concurrent chronic pain and depression in partial sciatic nerve ligation and completes Freund's adjuvant mouse models by inhibiting cAMP-PKA-CREB-BDNF signaling. Inhibiting PDE7A or activating cAMP-PKA-CREB-BDNF signaling are potential approaches for treating concurrent chronic pain and depression.

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INTRODUCTION

Approximately one-half of patients with chronic pain show symptoms of depression, and approximately 60% of depressed individuals complain of chronic pain (Agüera-Ortiz et al., 2011; Radat et al., 2013). Several studies on rodent models suggest that chronic pain, whether neuropathic or inflammatory, shares common disease mechanisms with depression, even if the 2 disorders occur separately through unique pathways (Kim et al., 2012; Brüning et al., 2015). The mechanistic overlap between the 2 disorders highlights the significance of studying them concurrently in appropriate preclinical models to identify therapeutic targets (Huang et al., 2016).

The phosphodiesterases (PDEs) comprise a large family of enzymes that catalyze the hydrolysis of cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) (Bender and Beavo, 2006). PDEs are vital regulators of cellular signal transduction with 11 known subfamilies (PDE1 to PDE11), which differ in structure, substrate specificity, and inhibitor sensitivity (Pearse et al., 2016). PDE2 (Wang et al., 2017; Liu et al., 2018b), PDE4 (Jindal et al., 2013; Zhang et al., 2022), PDE5 (Vieira et al., 2021; Bernardus Saayman et al., 2024), and PDE7 (Chłoń-Rzepa et al., 2018; Jankowska et al., 2020) inhibitors reportedly mitigate chronic pain or depression when these disorders occur individually. Hence PDE2, PDE4, PDE5, and PDE7 inhibition may reduce the comorbidity of chronic pain and depression. However, their role and mechanism of action in concurrent chronic pain and depression remain partially elucidated.

Among the 11 PDEs, PDE7 has 2 subtypes (PDE7A and PDE7B) and is reportedly the major PDE family responsible for cAMP hydrolysis in the central nervous system (CNS) (Chen et al., 2021b). PDE7 inhibition produces antinociceptive or anti-depression effects in the CNS (Chłoń-Rzepa et al., 2018; Jankowska et al., 2020). The cAMP, as a crucial secondary messenger, is a significant player and regulator of depression or the onset of chronic pain (Gao et al., 2022; Zhang et al., 2022). cAMP stimulates downstream cAMP-dependent protein kinase A (PKA), inducing the phosphorylation of cAMP response element binding protein (CREB), a transcription factor (Zhang et al., 2020; Cicek et al., 2022). CREB phosphorylation is significant in regulating gene transcription; it regulates brain-derived neurotrophic factor (BDNF) and markedly influences CNS function (Sun et al., 2021; Cicek et al., 2022).

The hippocampus is one of the main brain regions implicated in the occurrence and development of depression (Chan et al., 2016). Furthermore, the hippocampus plays an important role in pain (Vasic and Schmidt, 2017; Liu et al., 2018a). Hence, in this study, we examined the potential role of hippocampal PDEs in concurrent chronic pain and depression using chronic pain models, including partial sciatic nerve ligation (PSNL) and complete Freund's adjuvant (CFA) mice. In addition, we investigated the possible regulatory role of PDE7-mediated cAMP-PKA-CREB-BDNF signaling and neuroinflammation in the hippocampus. The results suggest a complex interaction between PDE7-cAMP signaling in the mediation of concurrent chronic pain and depression.

MATERIALS AND METHODS

Experimental Animals

Adult male C57BL/6J mice (Beijing Vital River Laboratory Animal Technology, Beijing, China) were housed in cages and subjected to controlled temperature and humidity under a 12-hour-light/-dark cycle in animal facilities free of specific pathogens at the Institute of Pharmacology at Shandong First Medical University. All animal experiments were approved by the Laboratory Animal Ethics Committee of Shandong First Medical University and performed according to the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication 80-23, revised 1996). The number of animals and their suffering were strictly minimized in each experiment.

Models of Concurrent Chronic Pain and Depression

To model concurrent neuropathic pain and depression, animals were anesthetized by i.p. injection of sodium pentobarbital (50 mg/kg). A small incision was made to expose the sciatic nerve of the left hind limb, and one-third to one-half of the nerve was tightly ligated using an 8-0 silk suture, as described by Zhang et al. (2022). Sham-operated animals underwent the same procedure, except that the sciatic nerve was not ligated after exposure. PSNL mice that showed no robust mechanical hypersensitivity (hind paw withdrawal threshold of >0.16 g) were excluded.

To model concurrent inflammatory pain and depression, mice were injected, as described by Duric and McCarson (2006), with 30 µL of CFA (Sigma-Aldrich, St. Louis, MO, USA) into the plantar surface of the left hind paw. Sham-treated animals were injected with 30 µL of saline. CFA mice with no robust mechanical hypersensitivity (hind paw withdrawal threshold of >0.16 g) were excluded.

Drug Administration and Testing Schedule

First, to observe the change in hippocampal PDE expression in both animal models, we measured the withdrawal thresholds of the 2 models using the von-Frey filament test at 4 weeks after ligation surgery or adjuvant injection. After the von-Frey test, the mice were subjected to the following behavioral tests in the indicated order: sucrose preference test (SPT), tail suspension test (TST), and forced swimming test (FST). After the FST, corresponding to 4 weeks after inducing chronic pain, the mice were decapitated, and contralateral (right) side hippocampi were collected for western blotting. Data from 40 male mice were analyzed. The procedures for each test are described below, and all tests were performed by investigators blinded to the animal treatment.

Subsequently, to examine the role of the PDE7 inhibitor, BRL50481, in concurrent chronic pain and depression, BRL50481 (10 mg/kg) or vehicle (0.1 mL/10 g) were orally administered to PSNL or CFA mice once daily for 14–33 days following surgery and injection; mice in the sham or saline groups were treated similarly with a vehicle as the naive control. Next, 4 weeks after the PSNL or CFA treatment, the mice were subjected to the following behavioral tests in the indicated order (i.e., 2 weeks following drug injection): von-Frey test, SPT, TST, and FST. The mice were then decapitated, and the contralateral (right) side hippocampi were collected for western blotting, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence staining analysis. The experiments involved 76 male mice. The BRL50481 was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China), dissolved in saline containing 1.2% dimethyl sulfoxide, and kept at 4°C before use. BRL50481 showed the potential to inhibit PDE7A expression at 1 mg/kg and significantly inhibited PDE7A expression at 10 mg/kg (data not shown). Thus, we used 10 mg/kg BRL50481.

To evaluate whether specifically downregulating hippocampal PDE7A can reverse chronic pain and depression, the effect of *PDE7A* knockdown achieved by intrahippocampal administration of adeno-associated virus (AAV) plasmid containing shRNA

targeting PDE7A on PSNL-induced concurrent chronic pain and depression was investigated. An intrahippocampal injection of AAV was conducted 7 days before sham or PSNL surgery. Subsequently, 5 weeks after injection (4 weeks after sham or PSNL surgery), the mice were subjected to the following behavioral tests in the indicated order: von-Frey test, SPT, TST, and FST. After the FST, the mice were decapitated, and the hippocampi were collected for western blotting. The experiments included 44 male mice.

Furthermore, to observe the role of cAMP signaling in concurrent chronic pain and depression, a cAMP activator, forskolin (2 mg/kg), or vehicle (0.1 mL/10 g) was orally administered to PSNL or CFA mice once daily (14–33 days) following surgery and injection; mice in the sham or saline group were treated similarly with a vehicle as the naive control. Next, 4 weeks after the PSNL or CFA treatment, the mice were subjected to the following behavioral tests in the indicated order (i.e., 2 weeks following drug injection): von-Frey test, SPT, TST, and FST. After the FST, the mice were decapitated, and their contralateral (right) side hippocampi were collected for western blotting. The experiments included 64 male mice. Forskolin was purchased from MedChemExpress (Shanghai, China), dissolved in saline containing 1.2% dimethyl sulfoxide, and kept at 4°C before use. We used 2 mg/kg forskolin based on a recent report (Chen et al., 2021a).

Von-Frey Test

As an index of pain tolerance, we measured the withdrawal threshold for the hind paw using von-Frey filaments (Zhang et al., 2022). Mice were housed in individually separate plastic boxes bottomed with metal mesh and allowed to acclimate for 45 minutes. Subsequently, von-Frey filaments (North Coast Medical, Gilroy, CA, USA) were pressed against the mid-planter surface of the hind paw; filaments were tested first in descending or ascending weight from 0.16 to 0.008 or 2 g. The threshold filament weight was defined as the minimal weight that caused responses such as lifting or licking of the ipsilateral hind paw at least thrice during 5 rounds of testing at 10-second intervals.

Depression- and Anxiety-Like Behaviors in the Animal Models

Sucrose Preference Test

The SPT was performed as previously described (Wang et al., 2024). The mice were deprived of water and food for 10 hours and provided ad libitum access to 2 bottles for 12 hours: one containing water and the other containing 1% sucrose solution. The bottle position was switched after 6 hours to prevent side preference. The bottles were weighed before and after the test, and sucrose preference was calculated as follows: sucrose preference (%) = sucrose intake (g) / [sucrose intake (g) + water intake (g)] × 100.

Tail Suspension Test

The TST was performed as previously described (Wang et al., 2020). Mice were suspended by their tail at 50 cm above a tabletop using adhesive tape placed approximately 1 cm from the tail tip. Animals were suspended for 6 minutes, and the time spent immobile during the last 4 minutes was determined as described using the DepressionScan Package (Clever Sys Inc., VA, USA).

Forced Swimming Test

The FST was performed as previously described (Wang et al., 2024). Each mouse was introduced into a cylinder (45 cm × 20 cm) containing tap water (25°C ± 2°C) to a depth of 25 cm. The mice were allowed to swim freely for 6 minutes, and the time spent immobile during the last 4 minutes was determined as described using the DepressionScan Package.

Western Blotting

After behavioral tests, animals were anesthetized through i.p. injection with pentobarbital (50 mg/kg) and decapitated. The contralateral (right) side hippocampus in the brain was removed, and the hippocampus was isolated and lysed in RIPA Cell/Tissue Rapid Lysis buffer (Solarbio, Beijing, China) containing protein phosphatase inhibitor (Solarbio) and the protease inhibitor phenylmethylsulfonyl fluoride (Solarbio) in the ratio of 100:1:1. The lysate was centrifuged at 13 000 × *g* for 10 minutes at 4°C, and total protein in the supernatant was added to Laemli buffer and boiled for 5 minutes. Equal amounts of protein (20 μg) were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Bio-Rad, Hercules, CA, USA) and transferred onto a polyvinylidene difluoride membrane (Millipore, St. Charles, MO, USA). The membrane was incubated overnight at 4°C with primary antibodies (diluted 1:1000) against the following proteins: PDE2A (Proteintech, Chicago, IL, USA), PDE4A (Proteintech), PDE4B (Cell Signaling Technology, Danvers, MA, USA), PDE4D (Abcam, Cambridge, UK), PDE5A (Abcam), PDE7A (Santa Cruz Biotechnology, Shanghai, China), PDE7B (Abcam), total PKA or phosphorylated PKA (p-PKA; Cell Signaling Technology), total CREB or phosphorylated CREB (p-CREB; Abcam), BDNF (Abcam), the p65 subunit of NF-kB (Cell Signaling Technology), or β-actin (Zsbio, Beijing, China). Subsequently, the membrane was incubated with a secondary antibody conjugated to horseradish peroxidase (1:2000; Zsbio). Next, incubation with electrochemiluminescence reagent (Absin) and the Amersham Imager 600 system (GE, Boston, MA, USA) was conducted, followed by quantification using the Image J V1.8.0 software (US National Institutes of Health, Bethesda, MD, USA).

ELISA

The hippocampus tissues were homogenized with an ice-cold phosphate buffered solution containing 1% phenyl methane sulfonyl fluoride. Lysates were repeatedly thawed and refrozen 3 times, and the supernatants were collected after centrifugation at 5000 ×g for 10 minutes. cAMP, cGMP, and TNF-α were contents determined using ELISA kits (Elabscience) following the instructions of the manufacturer; each sample was assessed in duplicates. A colorimetric reaction was conducted, and the absorbance at 450 nm was recorded using a multifunctional microplate reader (TECAN). Protein concentrations of samples were determined using enhanced BCA protein assay kits (Solarbio) following the manufacturer's instructions.

Immunofluorescence Staining

Immunofluorescence staining was performed as previously described (Hou et al., 2022). Whole brains were post-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer overnight, dehydrated through a graded ethanol series, and embedded in paraffin. The slices were dried in an oven at 60°C, dewaxed, rehydrated, and subjected to antigen retrieval. Samples were immersed in Triton X-100 and incubated at room temperature for 30 minutes. Subsequently, the sections were extensively washed in phosphate-buffered saline (PBS). The slices were blocked using 1% normal goat serum in PBS for 30 minutes at 37°C and incubated overnight at 4°C with anti-GFAP

antibody (1:500, Abcam), anti-Iba1 antibody (1:1000, Abcam), and anti-PDE7 antibody (1:200, Affanity). Next, after washing with PBS, the slices were incubated with the corresponding horseradish peroxidase–conjugated secondary antibody for 50 minutes. The slices were counterstained for 10 minutes with 4',6-diamidino-2-phenylindole staining solution to label the nucleus, washed with PBS, and mounted onto glass slides with resin mounting medium. Images were captured with a slice scanner (Pannoramic MIDI, Danjier, Jinan, China) and processed with Image-Pro-Plus software (version 6.0, Media Cybernetics, Bethesda, MD, USA).

Knockdown of Hippocampal PDE7A

The knockdown mouse PDE7A, AAV2/9-PDE7A-shRNA, and AAV2/9-shRNA NC with titers of $1\times10^{\wedge12}$ vg/mL were used (Hanbio, Shanghai, China). The targeting sequences for scrambled control-shRNA and PDE7A-shRNA were (TTCTCCGAACGTGTCACGTAA) and (CCATTTGGGTGTGAGTCCACTTTGT), respectively. Subsequently, 1.5 μL AAV was injected into the bilateral hippocampus (anterior-posterior position −2.0 mm, medial-lateral position +0.8 mm, 1.5 mm dorso-ventral from the bregma) at a flow rate of 0.25 μL/min using a 10-μL Hamilton syringe (Hamilton Co., USA). After the injection, the position of the needle was maintained for 5 minutes before it was slowly withdrawn.

Statistical Analyses

Data were analyzed using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). All quantitative data are expressed as the means ± SEM. Pairwise differences were assessed for significance using the Student *t* test, while differences among 3 or more groups were assessed using 1-way ANOVA with pairwise comparison using the Tukey–Kramer method for post-hoc comparisons. Differences were considered significant at $P<.05$.

RESULTS

PSNL- or CFA-Induced Chronic Pain, Depression, and Hippocampal PDE Expression

First, we determined the change in behavior and PDEs in both models following the schedule in Figure 1A. The induction of chronic pain in the PSNL or CFA model caused mechanical allodynia after 4 weeks ($P=.0001$ for PSNL and $P<0.0001$; Figure 1B) and reduced sucrose preference ($P=.0008$; Figure 1C) in the SPT, prolonging immobility time in the TST and FST ($P<.0001$; Figure

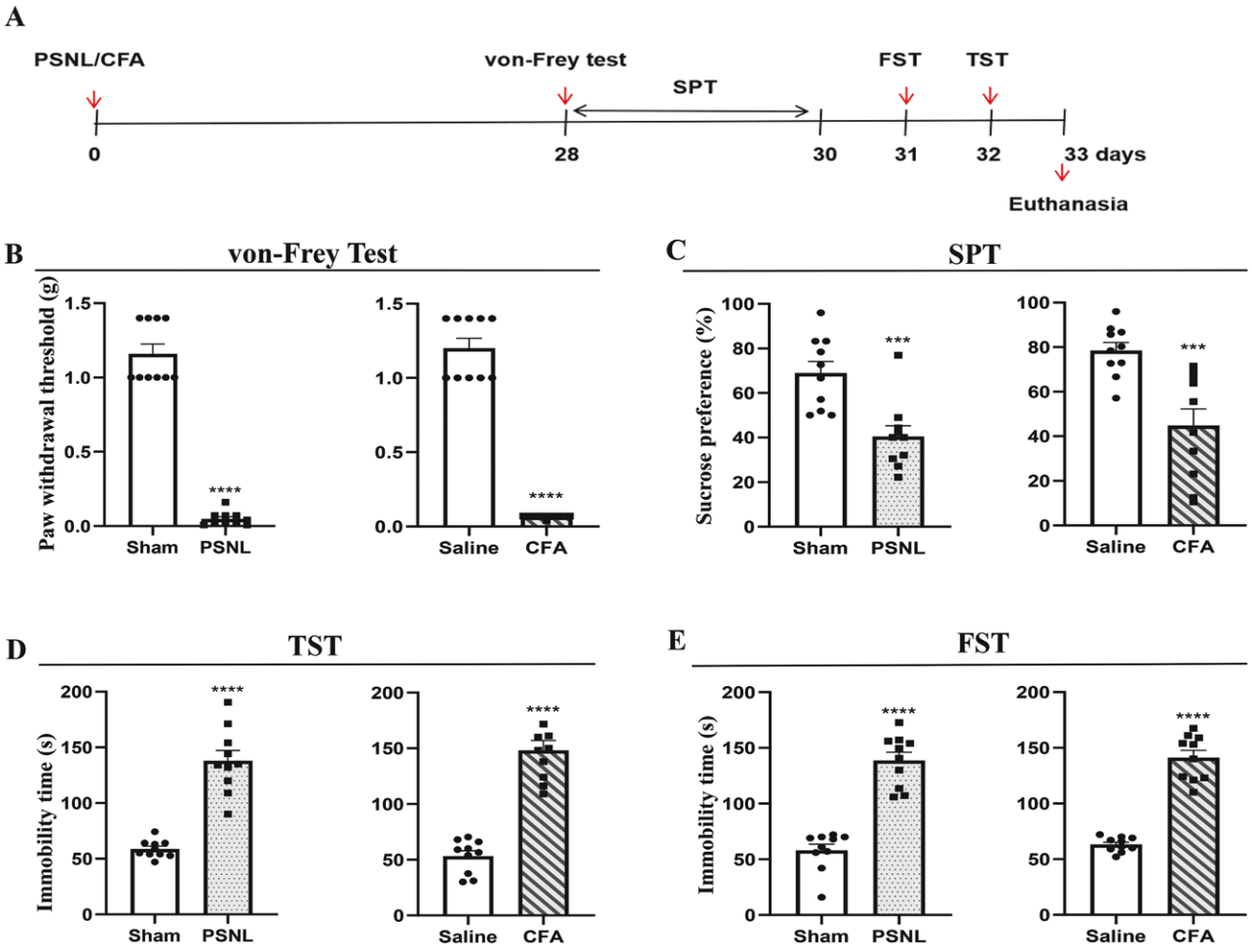

Figure 1. Partial sciatic nerve ligation (PSNL) or complete Freund's adjuvant (CFA) produced mechanical allodynia and depression-like behavior. (A) Schedule of modeling and testing. Mechanical allodynia and depression-like behavior was assessed in animals after PSNL or injection of CFA. (B) Mechanical allodynia was measured by the von-Frey test, (C) sucrose preference was measured by the sucrose preference test, and (D) immobility time was measured using the tail suspension test and (E) forced swimming test. Data shown are mean ± SEM (n = 10). ***$P<.001$, ****$P<.0001$ vs sham or saline.

1D and E). These findings confirm that the 2 animal models experienced concurrent chronic pain and depression.

We examined PDE2, PDE4, PDE5, and PDE7 expression levels in the hippocampus after the behavior tests using western blotting. Surgery or injection in the animal models upregulated hippocampal PDE4B ($P = .0282$ for PSNL and $P = .012$ for CFA; Figure 2C) and PDE7A ($P = .0005$ for PSNL and $P = .0476$ for CFA; Figure 2F) without affecting PDE2A ($P > .05$; Figure 2A), PDE4A ($P > .05$; Figure 2B), PDE4D ($P > .05$; Figure 2D), PDE5A ($P > .05$; Figure 2E), or PDE7B ($P > .05$; Figure 2G) levels. Furthermore, consistent with the ability of PDE4 and PDE7 to hydrolyze cAMP (Szczypka 2020), we found that cAMP, but not cGMP, exhibited significantly lower levels in the hippocampus of treated animals than in the hippocampus of sham-treated controls (Fig. 2H and I).

PSNL- or CFA-Induced Mechanical Hypersensitivity and Depression-Like Behavior Were Attenuated by BRL50481

The above results indicate that PDE4B or PDE7A may mediate concurrent chronic pain and depression by reducing hippocampal cAMP levels. PDE4 inhibitors have emetic-like effects; however, the inhibition of PDE7 showed a lack of emetic-like effects (Robichaud et al., 2002; García AM et al., 2014). Thus, we determined the role of PDE7 in PSNL- or CFA-induced mechanical hypersensitivity and depression-like behavior using BRL50481 (a specific PDE7 inhibitor) following the schedule in Figure 3A. BRL50481 (10 mg/kg, oral administration) partially reversed the effects of surgery or injection on mechanical hypersensitivity in the von-Frey test ($F_{(2,27)} = 142.6$, $P < .0001$ for PSNL, $F_{(2,27)} = 142.2$, $P < .0001$ for CFA; Figure 3B) and depression-like behaviors in the SPT ($F_{(2,27)} = 14.66$, $P < .0001$ for PSNL and $F_{(2,27)} = 10.52$, $P = .0004$ for CFA; Figure 3C), TST ($F_{(2,27)} = 55.61$, $P < .0001$ for PSNL and $F_{(2,27)} = 33.20$, $P < .0001$ for CFA; Figure 3D), and FST ($F_{(2,25)} = 11.97$, $P = .0002$ for PSNL and $F_{(2,27)} = 23.43$, $P < .0001$ for CFA; Figure 3E). In contrast, BRL50481 did not affect behavior in sham or saline mice (data not shown); the inhibitor partially reversed the hippocampal PDE7A upregulation induced by surgery or injection without affecting PDE7B levels (Figure 3F–G). In addition, similar to western blotting, PDE7A immunofluorescence intensity in the hippocampus was increased after PSNL in CA1, CA3, and DG and

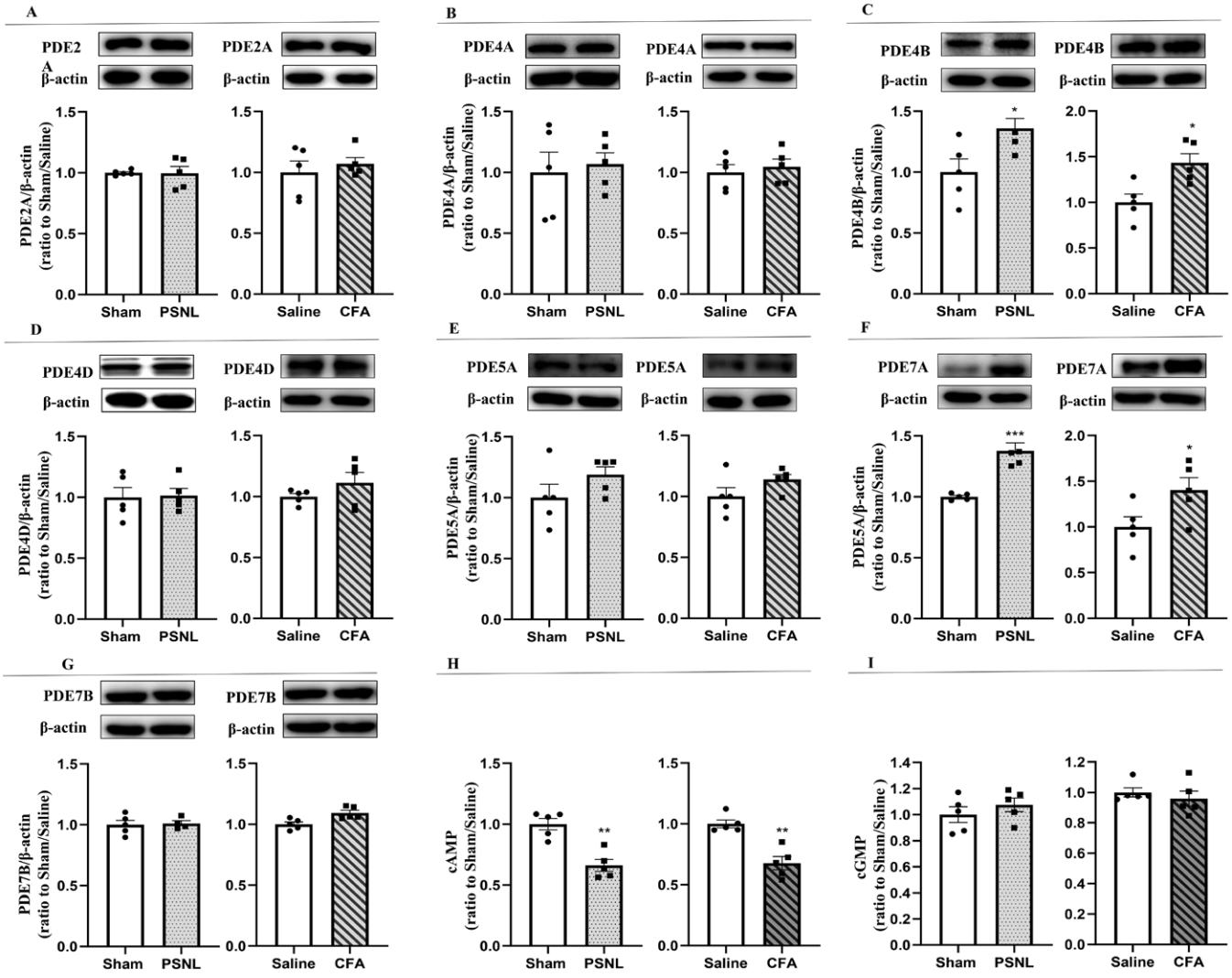

Figure 2. Partial sciatic nerve ligation (PSNL) or complete Freund's adjuvant (CFA) leads to increased phosphodiesterase 4B (PDE4B) and PDE7A and decreased cyclic AMP (cAMP) expression in mouse hippocampus. Mice were treated as described in the schedule of Figure 1. The expression of PDE2, PDE4, PDE5, and PDE7 in the hippocampus of mice subjected to PSNL or CFA were quantified by western blotting analysis (A–G), and the levels of cAMP and cyclic GMP (cGMP) were analyzed by ELISA assays (H–I) 33 days after surgery or injection. The optic densities of PDE2A, PDE4A, PDE4B, PDE4D, PDE5A, PDE7A, and PDE7B were normalized to those of sham (or saline) controls. Data shown are mean ± SEM (n = 5). *$P < .05$, **$P < .01$, ***$P < .001$ vs corresponding sham or saline.

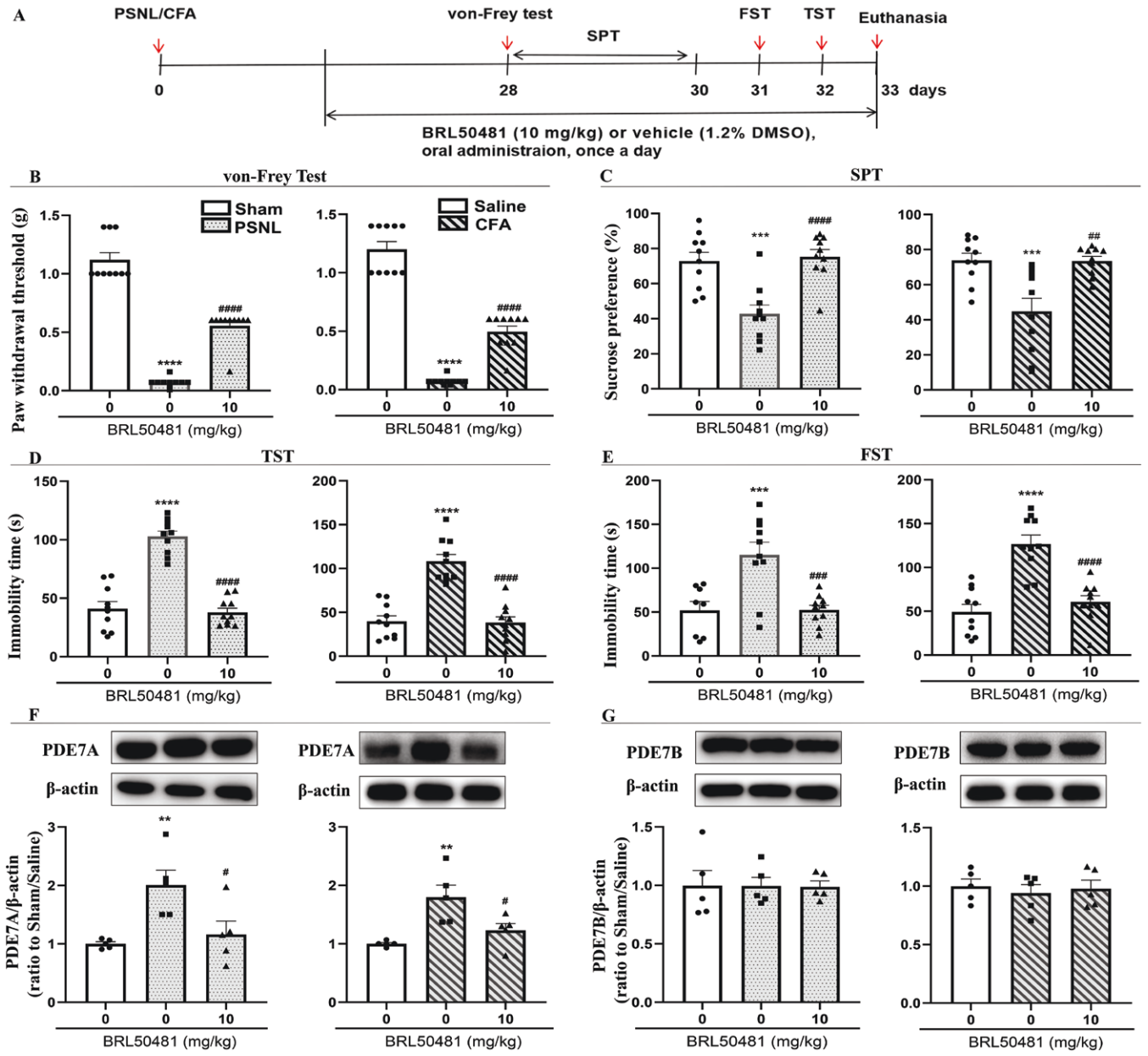

Figure 3. Oral administrated with phosphodiesterases 7 (PDE7) inhibitor BRL50481 reverses mechanical allodynia and depression-like behavior induced by partial sciatic nerve ligation (PSNL) or complete Freund's adjuvant (CFA) in mice. (A) Schedule of PDE7 inhibitor administration and testing. BRL50481 (10 mg/kg) or vehicle (0.1 mL/10 g) were orally administered to PSNL or CFA mice once daily for 14–33 days following surgery and injection; mice in the sham or saline groups were treated similarly with a vehicle as the control. Next, 4 weeks after the mold, (B) mechanical allodynia was measured by the von-Frey test, (C) sucrose preference was measured by the sucrose preference test, (D) immobility time was measured using the tail suspension test and (E) forced swimming test. After the behavioral tests, the PDE7A (F) and PDE7B (G) levels were measured by western blot. The protein levels were normalized to those of sham (or saline) controls. Data shown are mean ± SEM (n = 5). $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$ vs sham or saline. $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, $^{\#\#\#\#}P < .0001$ vs PSNL or CFA (no inhibitor).

was decreased after BRL50481 treatment ($F_{(2,6)} = 54.85$, $P = .0001$ for CA1; $F_{(2,6)} = 15.77$, $P = .0041$ for CA3; $F_{(2,6)} = 9.151$, $P = .0150$ for DG; Figure 4).

Knockdown of Hippocampal PDE7A With Recombinant AAV Reverses PSNL- or CFA-Induced Mechanical Hypersensitivity and Depression-Like Behavior

To confirm that specifically downregulating PDE7A in the hippocampus leads to chronic pain and depression, the effect of *PDE7A* knockdown through shRNA transfection on hind paw withdrawal thresholds and depression-like behavior was investigated. PDE7A expression in the hippocampus was significantly attenuated 40 days after intrahippocampal administration of PDE7A shRNA ($F_{(3,16)} = 15.07$, $P < .0001$ for PSNL, $F_{(3,16)} = 12.44$, $P = .0002$ for CFA; Figure 5B). PDE7B expression in the hippocampus was not changed after intrahippocampal administration of PDE7A shRNA (Figure 5C). Similarly, PDE7A knockdown caused a significant reduction in mechanical threshold ($F_{(3,19)} = 63.39$, $P < .0001$ for PSNL and $F_{(3,18)} = 59.93$, $P < .0001$ for CFA; Figure 5D) and depression-like behavior (Figure 5E–G) in PDE7A shRNA-treated PSNL or CFA mice.

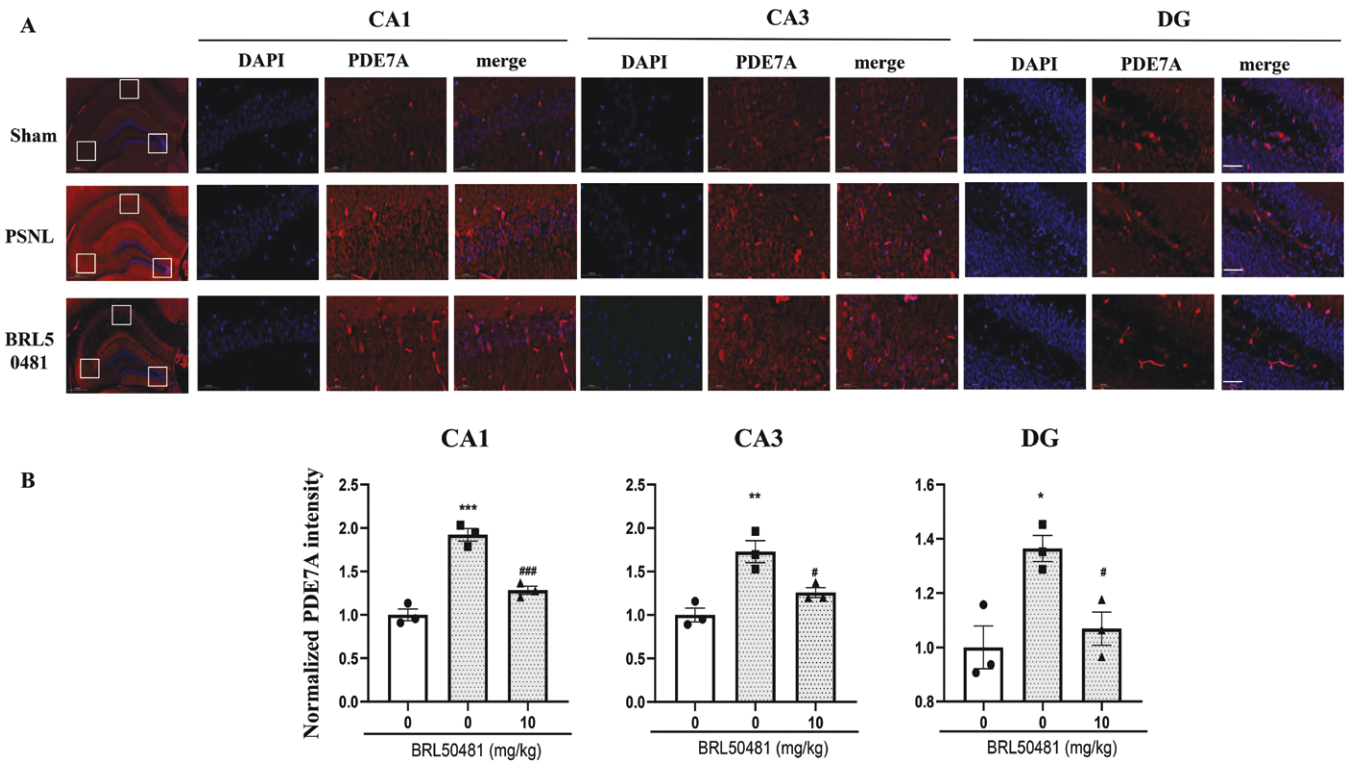

Figure 4. BRL50481 reverses phosphodiesterases 7A (PDE7A) immunofluorescence intensity in the hippocampus induced by partial sciatic nerve ligation in mice (PSNL). (A) Expression of PDE7A in hippocampal CA1, CA3, and DG regions based on immunohistochemistry. Scale bar, 50 μm. (B) Quantitative analysis of PDE7A in the hippocampus. Data shown are mean ± SEM (n = 3). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs sham or saline. $^{\#}P < .05$, $^{\#\#\#}P < .001$, vs PSNL (no inhibitor).

Downregulation of Hippocampal PD7A Ameliorated PSNL- or CFA-Induced Mechanical Hypersensitivity and Depression-Like Behavior via cAMP-PKA-CREB-BDNF Signaling

Mechanical hypersensitivity and depression-like behavior owing to PDE7A upregulation could be caused by the inhibition of hippocampal cAMP-PKA-CREB-BDNF signaling pathways. Therefore, we examined signaling in the hippocampus after BRL50481 treatment. The levels of cAMP and BDNF and the phosphorylation of PKA and CREB were significantly decreased in the hippocampus of PSNL ($F_{(3,16)} = 11.74$, $P = .0003$ for pPKA, $F_{(3,16)} = 10.27$, $P = .0005$ for pCREB, $F_{(3,16)} = 10.54$, $P = .0005$) or CFA ($F_{(3,16)} = 5.438$, $P = .009$ for pPKA, $F_{(3,16)} = 11.40$, $P = .0003$ for pCREB and $F_{(3,16)} = 5.764$, $P = .0072$) mice (Figure 6A–C). Similarly, the PSNL- or CFA-induced decrease were reversed after oral administration of BRL50481 (Figure 6A–C). Furthermore, the decrease in PKA and CREB phosphorylation and BDNF expression in PSNL or CFA mice was significantly reversed after the knockdown of hippocampal PDE7A (Figure 6D–F).

Furthermore, cAMP agonist forskolin (2 mg/kg) reversed PSNL- or CFA-induced decrease in PKA ($F_{(2,12)} = 37.81$, $P < .0001$ for PSNL and $F_{(2,12)} = 20.42$, $P = .0001$ for CFA) and CREB ($F_{(2,12)} = 13.74$, $P = .0008$ for PSNL and $F_{(2,12)} = 22.73$, $P < .0001$ for CFA) phosphorylation and BDNF ($F_{(2,12)} = 27.04$, $P < .0001$ for PSNL and $F_{(2,12)} = 12.11$, $P = .0013$ for CFA) expression (Figure 7B–D), similar to the effects of BRL50481. Forskolin also partially reversed the effects of surgery or injection on the mechanical hypersensitivity and depression-like behaviors of mice (Figure 7E–H). In contrast, forskolin did not affect behavior in sham or saline mice (data not shown). These findings suggest that PDE7A mediates concurrent chronic pain and depression via cAMP-PKA-CREB-BDNF signaling in the hippocampus.

BRL50481 Inhibited Neuroinflammation in the Hippocampus in the Mouse Model of Concurrent Chronic Pain and Depression

Next, we determined the change of neuroinflammation in PSNL model. The data showed that PSNL-induced increases in GFAP, and Iba1 were reversed by BRL50481 in the CA1 ($F_{(2,6)} = 138.0$, $P < .0001$ for GFAP and $F_{(2,6)} = 29.82$, $P = .0008$ for Iba1), CA3 ($F_{(2,6)} = 101.9$, $P < .0001$ for GFAP and $F_{(2,6)} = 8.491$, $P = .0178$ for Iba1), and DG ($F_{(2,6)} = 20.99$, $P = .0020$ for GFAP and $F_{(2,6)} = 11.23$, $P = .0094$ for Iba1) (Figure 8A–D).

Furthermore, PSNL mice also showed higher levels of IL-1β and NF-κB subunit p65 in the hippocampus, which were reversed by BRL50481 ($F_{(2,12)} = 11.09$, $P = .0019$ for IL-1β, $F_{(2,12)} = 9.247$, $P = .0037$ for p65; Figure 8E and F). These findings suggest that PDE7A mediates concurrent chronic pain and depression via neuroinflammation in the hippocampus.

DISCUSSION

Our experiments with 2 animal models of concurrent chronic pain and depression, one involving neuropathic pain and the other involving inflammatory pain, suggest comorbidity involves the upregulation of hippocampal PDE7A, which sharply reduces the levels of cAMP, dampening PKA-CREB-BDNF signaling and increasing neuroinflammation. We demonstrated that inhibiting

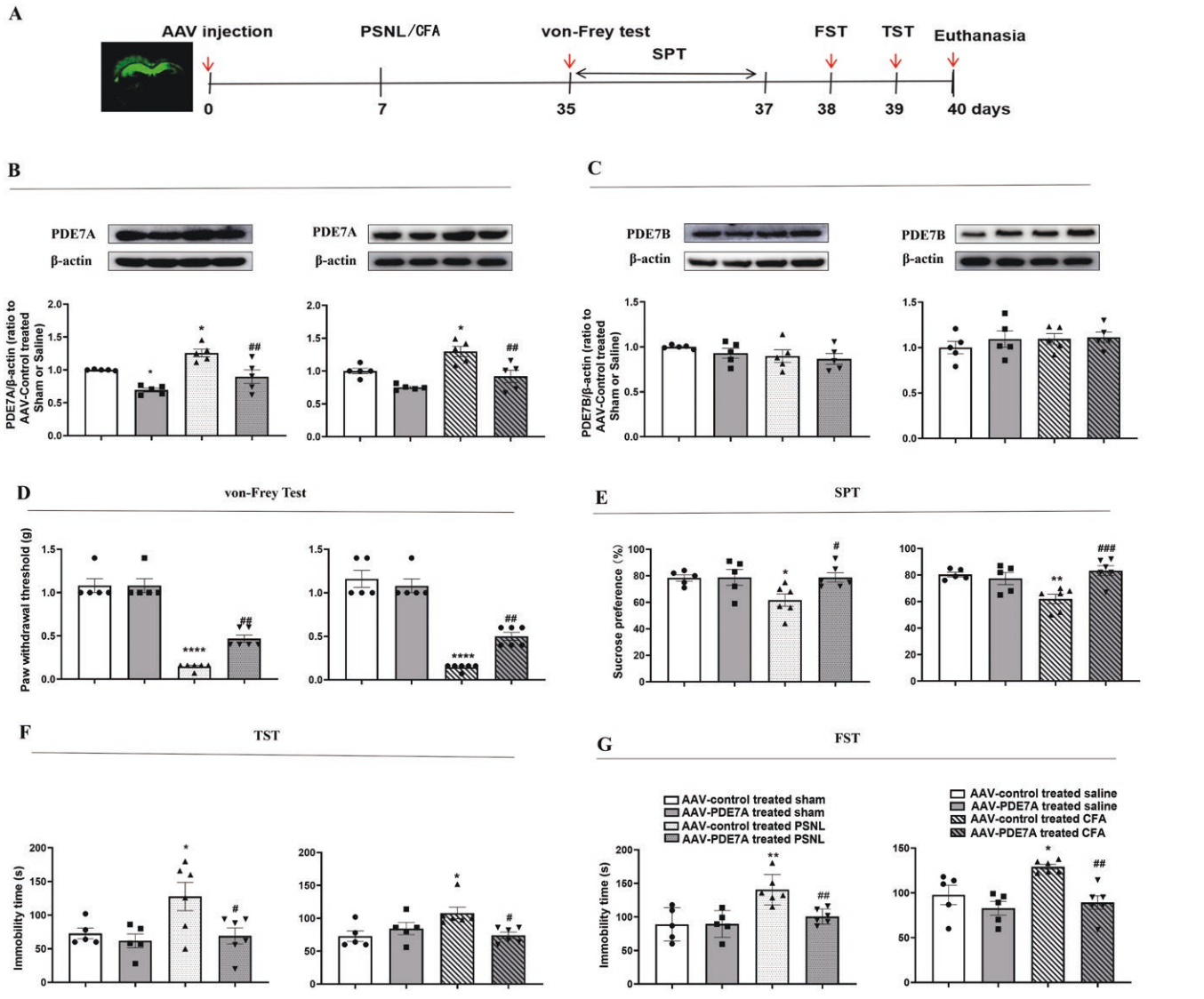

Figure 5. Knockdown of hippocampal phosphodiesterases 7A (PDE7A) with recombinant AAV reverses partial sciatic nerve ligation (PSNL)- or complete Freund's adjuvant (CFA)-induced mechanical hypersensitivity and depression-like behavior. (A) Schedule of AAV administration and testing. An intrahippocampal injection of AAV was performed 7 days before sham or PSNL surgery. Five weeks after injection (4 weeks after sham or PSNL surgery), the levels PDE7A (B) and PDE7B (C) were measured by western blot, mechanical allodynia was measured by the von-Frey test (D), sucrose preference was measured by the sucrose preference test (E), and immobility time was measured using the tail suspension test (F) and forced swimming test (G). Data shown mean ± SEM (n = 5 or 6). *P < .05, **P < .01, ****P < .0001 vs AAV-control-sham or -saline mice. #P < .05, ##P < .01 vs AAV-control-PSNL or -CFA mice.

PDE7A and neuroinflammation or stimulating cAMP-PKA-CREB-BDNF signaling may be effective therapies against concurrent chronic pain and depression.

PDE7 inhibition benefits various CNS disorders, such as Parkinson disease (Morales-Garcia et al., 2020), Alzheimer disease (Perez-Gonzalez et al., 2013), and multiple sclerosis (Medina-Rodríguez et al., 2013). A recent study demonstrated that PDE7 inhibition mitigates chronic pain and depression when these disorders occur individually. Similarly, a recently demonstrated PDE4B/PDE7A inhibition reduces the immobility time of mice in the FST (Jankowska et al., 2020). Furthermore, PDE4B/PDE7A inhibition can exert anti-inflammatory and analgesic effects (Chłoń-Rzepa et al., 2018). Here, we demonstrated that oral administration of a selective PDE7 inhibitor, BRL50481 (N, N, 2-trimethyl-5-nitrobenzenesulfona-30 mide), ameliorated PSNL- or CFA-induced pain and depression in mice. In addition, BRL50481 reversed PSNL- or CFA-induced increases in hippocampal PDE7A expression. These data suggest that PDE7 is involved in regulating concurrent chronic pain and depression.

The PDE7 family includes 2 subtypes, namely PDE7A and PDE7B. Here, we demonstrated the expression of PDE7 isoforms in the hippocampus of PSNL or CFA mice treated with the PDE7 inhibitors. PSNL and CFA increased PDE7A expression but not PDE7B expression. BRL-50481 is the most common PDE7 inhibitor, with IC_{50} values of 0.15 and 12.1 2 μM for PDE7A and PDE7B, respectively (Smith et al., 2004). We showed that 10 mg/kg BRL-50481 decreased PDE7A expression but not PDE7B expression. PDE7A may be the PDE7 isoform mediating the antinociceptive and anti-depression effects of PDE7 inhibitors. Furthermore, it

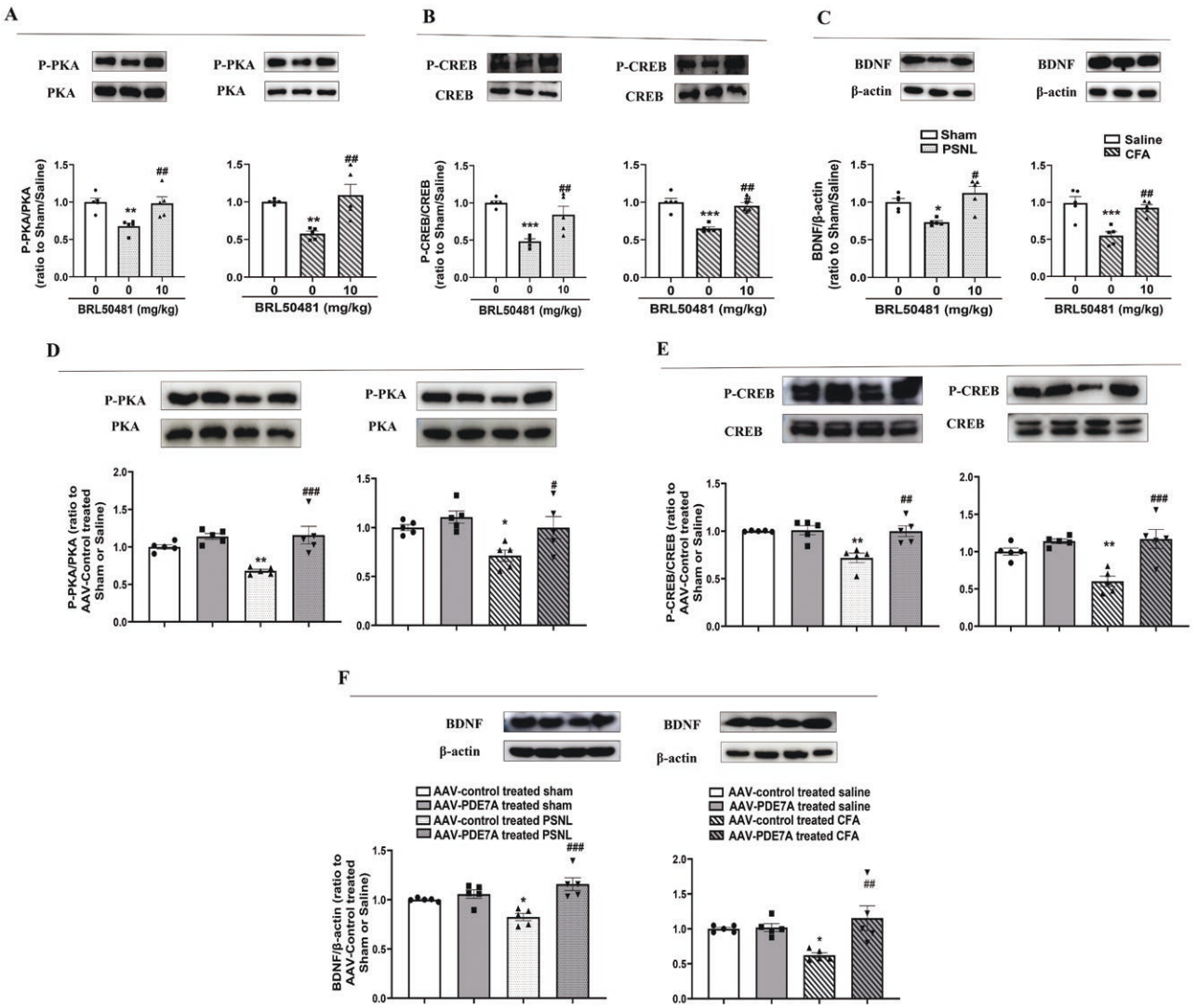

Figure 6. Downregulation of hippocampal phosphodiesterases 7A (PD7A) reverses partial sciatic nerve ligation (PSNL)- or complete Freund's adjuvant (CFA)-induced inhibition of cAMP-protein kinase A (PKA)-cAMP response element binding protein (CREB)-brain-derived neurotrophic factor (BDNF) signaling. (A–C) Mice were treated as described in the schedule of Figure 3. Phosphorylation of PKA and CREB and BDNF levels in the hippocampus of PSNL or CFA mice were measured by western blot (D–F). Mice were treated as described in the schedule of Figure 5. Phosphorylation of PKA and CREB and BDNF levels in the hippocampus of PSNL or CFA mice were measured by western blot. Data shown mean ± SEM (n = 5 or 6). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs vehicle or AAV-control-sham or -saline mice. $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$ vs vehicle or AAV-control-PSNL or -CFA mice.

is important to identify the cell type expressing PDE7A, and we intend to conduct future studies to investigate the role of neurons and glial cells.

Unlike PDE7, PDE2 and PDE5 have been implicated in animal models of pain or depression when these disorders occur individually (Wang et al., 2017; Liu et al., 2018b; Szczypka 2020; Megat et al., 2022), However, in our data, hippocampal PDE2A and PDE5A exhibited no changes in expression following PSNL or CFA. In contrast hippocampal PDE4B expression was increased following PSNL or CFA. PDE4 inhibitors modify the duration of anesthesia induced by the combination of ketamine and xylazine (Robichaud et al., 2002). Unlike the inhibition of PDE4, PDE7 inhibition has been shown to lack emetic-like effects (García AM et al., 2014). Thus, we investigated the role of PDE7 in both models in this study. Nevertheless, many studies have examined the correlation of PDE4 and the pathogenesis of CNS diseases, including depression and chronic pain (Jindal et al., 2013; Zhang et al., 2022). Further studies are required for a comparative analysis of the antinociceptive and antidepressant effects of PDE 4B and 7A inhibitors.

PDE7A hydrolyzes cAMP in the CNS (Chen et al., 2021). As observed in the present study, PSNL or CFA induced a decrease in cAMP but not cGMP. The cAMP activator, forskolin, ameliorated PSNL- or CFA-induced pain and depression in mice, indicating that the therapeutic potential of inhibiting PDE7A is related to upregulating cAMP signaling. Similarly, our study suggests the therapeutic potential of stimulating PKA-CREB-BDNF signaling. These results are consistent with those of other studies, indicating that activation of the cAMP-CREB-BDNF signaling pathway exerts neuroprotective and antidepressant effects in mouse models of depression (Liu et al., 2018b). These data imply that PKA-CREB-BDNF is a downstream target of cAMP signaling and can mitigate chronic pain and depression whether these disorders occur individually or concurrently.

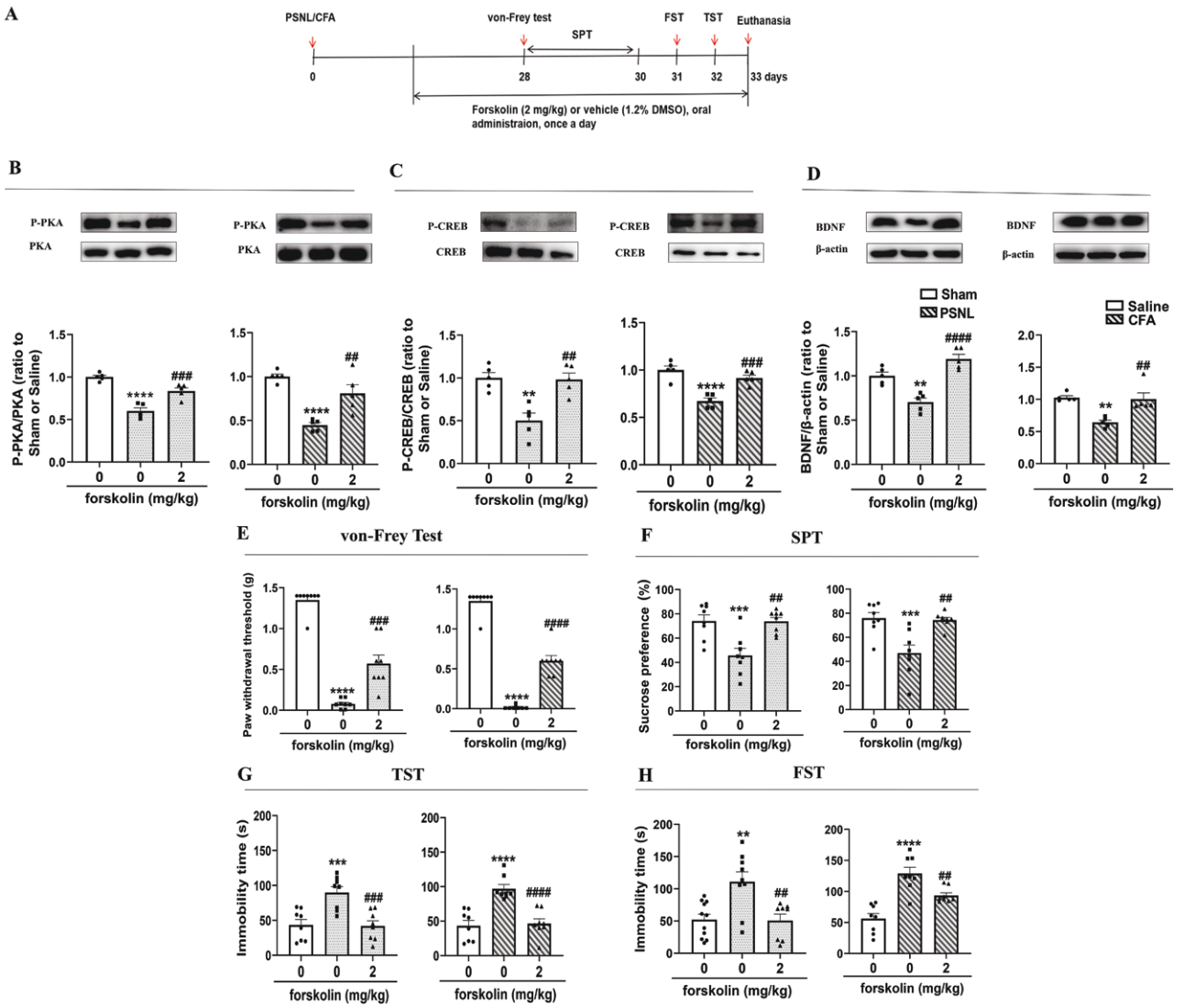

Figure 7. Oral administration of cAMP activator forskolin reverses PSNL- or CFA-induced inhibition of PKA-CREB-BDNF signaling.(A) Schedule of forskolin administration and testing. Forskolin (2 mg/kg) or vehicle (0.1 mL/10 g) was orally administered to PSNL or CFA mice once daily for 14–33 days following surgery and injection; mice in the sham or saline groups were treated similarly with a vehicle as the control. pPKA (B), pCREB (C), and BDNF (D) levels were measured by western blot. Mechanical allodynia was measured by the von-Frey test (E), sucrose preference was measured by the sucrose preference test (F), immobility time was measured using the tail suspension test (G) and forced swimming test (H). Data shown are mean ± SEM (n = 10 for behavior test and n = 5 mice for western blot). $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$ vs sham or saline. $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, $^{\#\#\#\#}P < .0001$ vs PSNL or CFA (no inhibitor).

Hippocampal neuroinflammation, potentially mediated by hyperactivation of astrocytes and microglia, has been closely linked to chronic pain and depression (Hu et al., 2017). As observed in this study, PSNL-induced comorbidity of chronic pain and depression was associated with the activation of astrocytes, as well as with microglia in hippocampus. PSNL also induces increased IL-1β levels and p65 phosphorylation in the hippocampus. This is consistent with a report that spinal nerve ligation-induced depression comorbidity of a chronic pain rat model also showed neuroinflammation changes in the hippocampus (Hu et al., 2017). These data indicate that inhibiting neuroinflammation in the hippocampus can mitigate chronic pain and depression whether these disorders occur individually or concurrently. Furthermore, our data demonstrated that inhibiting PDE7 can reverse neuroinflammation, indicating that neuroinflammation is a downstream target of PDE7-cAMP signaling.

In this study we focused specifically on the hippocampus because of its strong association with chronic pain and/or depression (Yalcin et al., 2014; Fasick et al., 2015). The CA1, CA2, and DG regions of the hippocampus have been implicated in neuroinflammation (Cui et al., 2020). We found that PSNL mice showed neuronal inflammation and PDE7A increase in these regions, which were ameliorated by BRL50481.

We demonstrated a distinct mechanism of PSNL- or CFA-induced comorbidity of chronic pain and depression regulated by PDE7-mediated intracellular signaling. Both models induced PDE7A expression, leading to decreases in cAMP levels and PKA-CREB-BDNF signaling activity. This caused increased proinflammatory cytokines levels, such as IL-1β and phosphorylation of NF-kB and activation of glia cells in the hippocampus, resulting in chronic pain and depression. Conversely, PDE7A inhibition activated cAMP-PKA-CREB-BDNF signaling, suppressed IL-1β levels, NF-kB

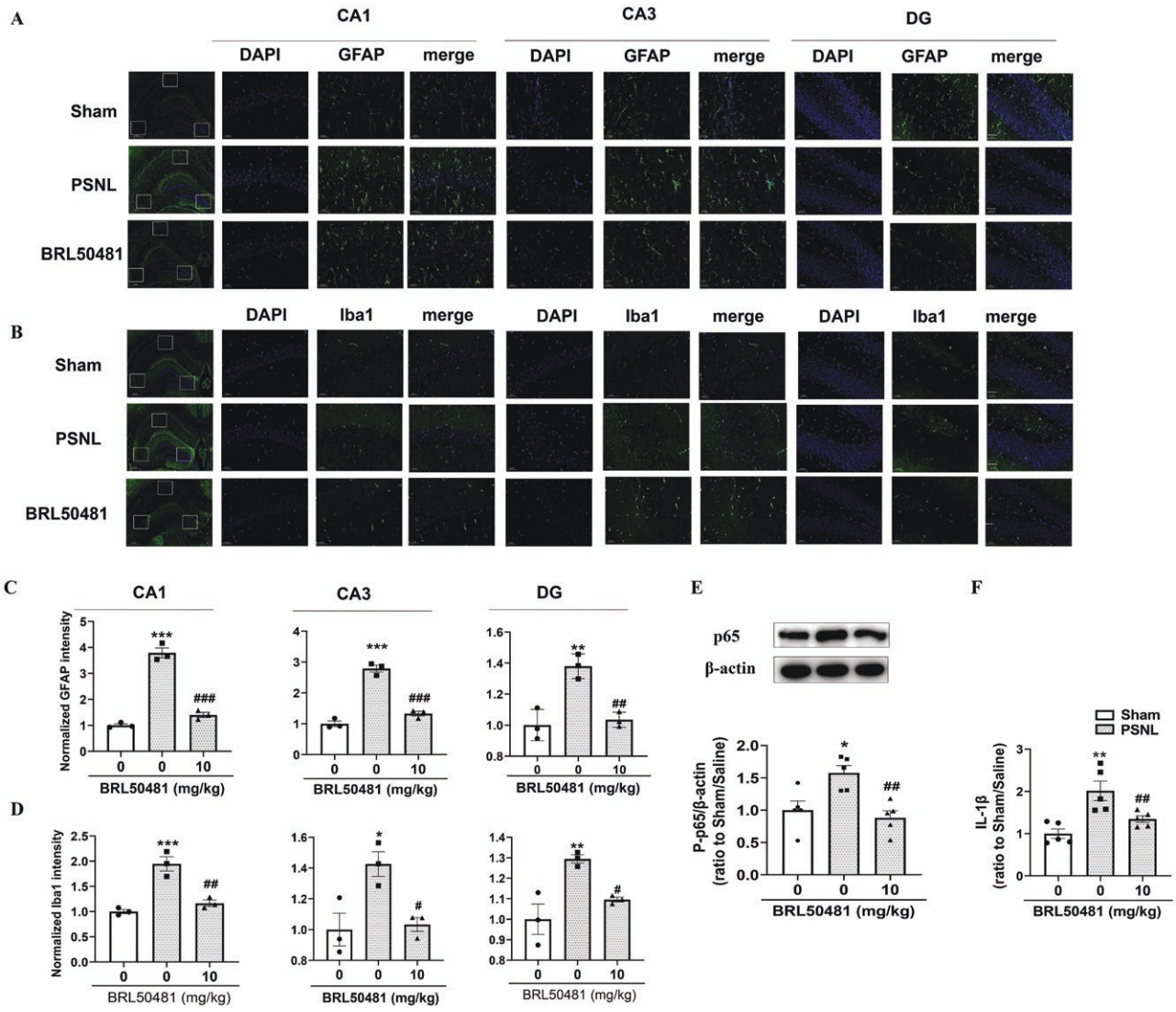

Figure 8. BRL50481 inhibited neuroinflammation in the PSNL mice.Mice were treated as described in the schedule of Figure 3. (A and B) Representative micrographs by immunofluorescence for GFAP and Iba-1 in the hippocampal CA1, CA3, and DG region. Scale bar, 50 μm. Quantitative analysis of GFAP (C) and Iba-1 (D). Levels of IL-1β (E) and p65 (F) in hippocampus. Data shown mean ± SEM of 3–5 mice per condition. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs sham. $^{\#\#}P < .01$ vs PSNL (no inhibitor or agonist).

phosphorylation, and activation of glial cells in the hippocampus, suppressing chronic pain and depression. This study clarifies the common disease pathways underpinning chronic pain and depression and facilitates the development of novel treatments.

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Interest Statement

The authors declare that they have no conflicts of interest.

Data Availability

The article contains original manuscripts. For further inquiries, please contact the corresponding author directly.

Author Contributions

Shi-cai Chen (Data curation [Equal], Formal analysis [Equal], Investigation [Supporting], Methodology [Supporting], Software [Lead], Validation [Lead], Visualization [Equal], Writing—original draft [Lead], Writing—review and editing [Supporting]), Yanhan Chen (Methodology [Supporting], Validation [Supporting], Writing—original draft [Supporting]), Yan Song (Methodology [Supporting], Validation [Supporting]), Shu-hua Zong (Validation [Supporting]), Ming-xia Wu (Validation [Supporting]), Wei Wang (Methodology [Supporting], Validation [Supporting]), Hao Wang (Data curation [Supporting], Validation [Supporting]), Feng Zhang (Methodology [Supporting], Validation [Supporting]), Yan-Meng Zhou (Supervision [Supporting], Validation [Supporting]), Haiyang Yu (Validation [Supporting]), Han-Ting Zhang (Data curation [Supporting], Funding acquisition [Equal], Methodology [Supporting], Project administration [Supporting], Resources [Supporting], Supervision [Supporting], Visualization [Equal], Writing—review and editing [Supporting]), and Fang-fang Zhang (Conceptualization [Lead], Data curation [Equal], Formal

analysis [Equal], Funding acquisition [Lead], Investigation [Lead], Methodology [Equal], Project administration [Lead], Resources [Equal], Software [Supporting], Supervision [Lead], Validation [Supporting], Visualization [Equal], Writing—original draft [Supporting], Writing—review and editing [Lead]).

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