αCaMKII in the lateral amygdala mediates PTSD-Like behaviors and NMDAR-Dependent LTD

Shuming An a,1, Jiayue Wang a,1, Xuliang Zhang a,1, Yanhong Duan a, Yiqiong Xu b, Junyan Lv a, Dasheng Wang a, Huan Zhang a, Gal Richter-Levin c, Oded Klavir a, Buwei Yu b,*, Xiaohua Cao a,1,

a Key Laboratory of Brain Functional Genomics, Ministry of Education, School of Life Sciences, East China Normal University, 3663 North Zhongshan Road, Shanghai, 200062, China
b Department of Anesthesiology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, China
c Sagol Department of Neurobiology, University of Haifa, Haifa, 31905, Israel

A R T I C L E   I N F O
Keywords:
Posttraumatic stress disorder (PTSD)
αCaMKII
Cued fear conditioning
LTD
Anxiety
Amygdala

A B S T R A C T
Post-traumatic stress disorder (PTSD) is a psychiatric disorder that afflicts many individuals. However, its molecular and cellular mechanisms remain largely unexplored. Here, we found PTSD susceptible mice exhibited significant up-regulation of alpha-Ca2+/calmodulin-dependent kinase II (αCaMKII) in the lateral amygdala (LA). Consistently, increasing αCaMKII in the LA not only caused PTSD-like behaviors such as impaired fear extinction and anxiety-like behaviors, but also attenuated N-methyl-D-aspartate receptor (NMDAR)-dependent long-term depression (LTD) at thalamo-lateral amygdala (T-LA) synapses, and reduced GluA1-Ser845/Ser831 dephosphorylation and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) internalization. Suppressing the elevated αCaMKII to normal levels completely rescued both PTSD-like behaviors and the impairments in LTD, GluA1-Ser845/Ser831 dephosphorylation, and AMPAR internalization. Intriguingly, deficits in GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization were detected not only after impaired fear extinction, but also after attenuated LTD. Our results suggest that αCaMKII in the LA may be a potential molecular determinant of PTSD. We further demonstrate for the first time that GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization are molecular links between fear extinction and LTD.

1. Introduction

Posttraumatic stress disorder (PTSD) is one of the most prevalent and debilitating psychiatric illnesses worldwide (Etkin and Wager 2007; Yehuda et al., 2015). Flashbacks (reliving of natural disaster, military combat, or physical assault-associated traumatic events), impaired fear extinction, increased anxiety, and avoidance of trauma associated stimuli are the typical symptoms of PTSD (Clausen et al., 2020; Li et al., 2020). Accumulating evidence indicates that the pathophysiology of PTSD critically involves lateral amygdala (LA) dysregulation (Castro-Gomes et al., 2016; Ousdal et al., 2020; Fenster et al., 2018). Specifically, PTSD patients show exaggerated LA activity (Etkin and Wager 2007) and smaller than normal LA volume (Morey et al., 2020; Ousdal et al., 2020). Furthermore, LA lesions cause the changes in fear- and anxiety-related behaviors (Fanselow and Gale 2003; Nader et al., 2001; Davis 1992). However, the cellular and molecular mechanisms through which the LA modulates PTSD are largely unknown.

Besides anxiety-related phenotypes, impaired fear extinction is one of the hallmark symptoms of PTSD (Michopoulos et al., 2014; Yehuda et al., 2015), and fear extinction is the basis for psychological exposure therapy (Milad and Quirk 2012). Recent studies have uncovered a link between fear extinction, long-term depression (LTD), and glutamatergic neurotransmission in the LA. Of note, LTD has been implicated in fear extinction (Bennett et al., 2017). Specifically, N-methyl-D-aspartate (NMDA) GluN2B receptor antagonist can abolish both LTD at thalamo-lateral amygdala (T-LA) synapses and fear extinction (Dalton et al., 2012). Similarities occur in the hippocampus with the deletion of kinesin superfamily proteins (KIFs) 21B (Morikawa et al., 2018), while...
aquaporin-4 deficiency facilitates both fear extinction and NMDAR-dependent hippocampal LTD (Wu et al., 2017). Furthermore, optogenetic delivery of LTD stimuli to the auditory input to the LA facilitates cued fear extinction (Nabavi et al., 2014). Taken together, these findings indicate that there may be a link between LTD and fear extinction.

Previous studies suggest that NMDAR-dependent α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) internalization is involved in fear extinction (Dalton et al., 2008; Kim et al., 2007; Lin et al., 2010; Bai et al., 2014). Specifically, disruption of AMPAR internalization impairs fear extinction (Dalton et al., 2008; Kim et al., 2007), whereas enhanced AMPAR internalization facilitates fear extinction (Lin et al., 2010; Bai et al., 2014). It is worth noting that AMPAR internalization also participates in LTD (Brebner et al., 2005; Collingridge et al. 2004). Hence, we hypothesized that AMPAR internalization may be a link between fear extinction and LTD.

PTSD-like behaviors include impaired fear extinction and heightened anxiety (Régue et al., 2019; Arnold et al., 2016). Here, using a behavioral profiling approach (Ardi et al., 2016), we therefore identified the PTSD-susceptible mice by cued fear extinction deficits and anxiety-like behaviors following trauma exposure. Notably, increased alpha-Ca^2+ /calmodulin-dependent kinase II (αCaMKII) was detected in the LA of PTSD susceptible mice. αCaMKII is an abundant synaptic protein, which plays a crucial role in memory formation (Irivine et al., 2006) and long-term potentiation (LTP) (Lisman et al. 2002). However, whether αCaMKII participates in the processing of fear extinction and LTD remains to be elucidated.

To determine whether increased αCaMKII causes PTSD-like behaviors, we employed an inducible and reversible chemical-genetic technique to temporally and spatially manipulate αCaMKII level in the forebrain of αCaMKII-F89G TG mice, as well as using adeno-associated viral (AAV) vectors to elevate αCaMKII specifically in the LA of C57BL/6J mice. Consistently, the up-regulation of αCaMKII induced PTSD-like behaviors including cued fear extinction deficits and anxiety-like behaviors, which could be reversed by suppressing elevated αCaMKII to normal levels. In addition, we demonstrated that GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization are the links between cued fear extinction and NMDAR-dependent LTD at T-LA synapses. These findings provide novel insights into the mechanisms underlying PTSD and new therapeutic targets.

2. Material and methods

2.1. Animals

2.1.1. Biochemical characterizations of αCaMKII-F89G TG mice

αCaMKII-F89G TG mice were donated by Dr. Tsiens’s lab (Wang et al., 2003). Mutant αCaMKII-F89G was generated with a silent mutation (i.e. replacing the Phe-89 with Gly in αCaMKII), so that the αCaMKII-F89G TG mice, as well as using adeno-associated viral (AAV) vectors to elevate αCaMKII specifically in the LA of C57BL/6J mice. Consistently, the up-regulation of αCaMKII induced PTSD-like behaviors including cued fear extinction deficits and anxiety-like behaviors, which could be reversed by suppressing elevated αCaMKII to normal levels. In addition, we demonstrated that GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization are the links between cued fear extinction and NMDAR-dependent LTD at T-LA synapses. These findings provide novel insights into the mechanisms underlying PTSD and new therapeutic targets.

2.2. Behavior experiments

2.2.1. Behavioral profiling for identification of PTSD susceptible mice

We applied a behavioral profiling approach (Ardi et al., 2016) to identify PTSD susceptible mice in either undergraduate trauma (UWT)-exposed mice (Ritov et al. 2016) or four conditioned stimulus/unconditioned stimulus (4-CS/US)-exposed mice (Milad and Quirk 2011; Mahan and Ressler 2012; Radulovic et al. 2019; Borghans and Homberg 2015; Debiec et al., 2011; Fenster et al., 2015; It et al., 2014).

In detail, the C57BL/6J mice were randomly divided into three groups: control group (n = 12), UWT-exposed group (n = 23) and 4-CS/US-exposed group (n = 23). The control mice were kept in home cages for 4 weeks without any treatment. The UWT-exposed mice were individually allowed to swim freely for 5 s in a water-filled plastic tank, then submerged under water for 35 s using a metal net, next kept in their home cages for 4 weeks (Ritov et al. 2016; Ardi et al., 2016). The 4-CS/US-exposed mice were individually placed in the chamber and allowed to explore the environment freely for 2 min, and then exposed to the conditioned stimulus (CS: 75 dB sound at 2800 Hz) for 30 s. At the last 2 s of tone stimulus, the unconditioned stimulus (US: 0.50 mA footshock, 2 s) was delivered. After 4-CS/US pairings with 2 min intertrial interval, mice were kept in the test chamber for 2 min and then transferred to their home cages for 4 weeks.

Three groups were subjected to the open field (OF), light/dark (LD), water associated zero maze (WAZM), and fear conditioning and extinction paradigms. Freezing behavior was monitored by Freeze Frame system (Coulbourn Instruments, USA).

We calculated six parameters: two parameters represent the level of locomotor activity and four parameters represent anxiety-like performances in the four paradigms. To create the behavioral profiles, firstly we referred to the performance of the control group as the behavior of the normal population and determined the distribution of values in the control group. Standard deviations were used to calculate the upper and lower “cut-off values” for each selected parameter. Secondly, the performance of each mouse in the UWT-exposed group or 4-CS/US-exposed group was compared to the distribution curve of the control group. Mice were considered PTSD susceptible if they exhibited values that are under the lower cut-off value or above the upper cut-off value in at least four out of the six parameters. “Cut-off values” of six parameters: time spent in the center of the OF test, 560.32 ± 34.52 s; time spent in the light box in the LD test, 788.60 ± 58.92 s; time spent in the open arms in the OM test, 111.43 ± 8.88 s; freezing percentage in the last day of cued fear extinction, 31.98% ± 3.91%; total distance in the OF test, 7059.99 ± 427.80 cm; total distance in the LD test, 9124.67 ± 220.50 cm.

2.2.2. Cued fear extinction

Four weeks after 4-CS/US pairings or 24 h after the 1-CS/US pairing, each mouse was placed in a novel chamber and monitored for 2 min (in the absence of the tone). For the recall test, the cued freezing response to a 3 min tone (75 dB sound at 2800 Hz) without footshock was measured. Then, four cued fear extinction trials were conducted like the recall test in the next 4 following days. Data were presented as mean ± s.e.m. Two-way ANOVA was used for statistical analysis.

S. An et al.
2.2.3. Open field

As described previously (Yan et al., 2015), briefly, each mouse was placed in an acrylic open-field chamber (27 cm long × 27 cm wide × 38 cm high) for 30 min. Total moving distance, time spent in the center area, and number of rearing were measured using a TRU-SCAN DigBahv-locomotion Activity Video Analysis System (Coulbourn Instruments, USA). Data were presented as mean ± s.e.m. One-way ANOVA was used for statistical analysis in Figs. 1C and 2C and Mann Whitney test in Fig. S2A.

2.2.4. Light/dark

The box (27 cm long × 27 cm wide × 38 cm high) was divided into two equal zones - light zone and dark zone. The light zone was painted white and illuminated by white light while the dark zone was painted black and not illuminated. These two zones were connected by a door in the middle divider, and the mice could shuttle freely between the two zones. Total distance and time spent in the light zone were delineated by the TRU-SCAN DigBahv-locomotion Activity Video Analysis System (Coulbourn Instruments, USA) for 30 min. Data were presented as the mean ± s.e.m. One-way ANOVA was used for statistical analysis in Figs. 1D and 2D.

2.2.5. Water-associated zero maze (WAZM)

Experimental protocol and device were as described previously.
This device was composed of an annular platform and a plastic bucket. The annular platform was divided into four equal quadrants: similar to two opposed open arms and two opposed closed arms. The plastic bucket was full of water 40 cm deep. After 5 min habituation, a mouse was put into one of the open arms facing the closed arm for 5 min. The time spent in the open arms vs. closed arms was measured by ANY-maze system (USA, Stoelting). Data were presented as mean ± s.e.m. One-way ANOVA was used for statistical analysis.

### 2.2.6 Elevated plus maze

The apparatus consists of two opposed open arms (30 cm × 5 cm), two opposed closed arms (30 cm × 5 cm) and one open square (5 cm × 5 cm) in the center, which was elevated above the floor (50 cm). Each mouse was placed in the center of the plus maze facing an open arm and allowed to explore for 5 min. The time spent and moving distances in
open and closed arms were automatically recorded by the ANY-maze system (USA, Stoelting). Data were presented as mean ± s.e.m. One-way ANOVA was used for statistical analysis.

2.2.7. Sensitivity to foot shock
This test was performed according to the methods as published (Duan et al., 2015). Mice were individually placed in the conditioning chamber to receive 1 s shocks of gradually increasing current intensity by an increment of 0.01 mA (flinching, 0.05–0.1 mA; vocalization, 0.1–0.2 mA; jumping, 0.45–0.6 mA) with 20 s intervals. The minimum current required to elicit flinching, vocalization and jumping in mice were measured. Data were presented as mean ± s.e.m. Mann Whitney test was used for statistical analysis.

2.3. Animal surgery
To elevate αCaMKII specifically in the LA of C57BL/6J mice, we injected pAAV-TRE-αCaMKII-P2A-EGFP-CMV-rTA (AAV-αCaMKII) or pAAV-TRE-P2A-EGFP-CMV-rTA (AAV-control) virus (2.45 × 10¹² and 2.38 × 10¹² vector genomes/ml, respectively, Obio Technology, China)
bilaterally into the LA (AP, −1.60 mm; ML, ±3.35 mm; DV, −4.80 mm) of C57BL/6J mice. After the injection, mice were returned to the home cages to recover for one month before experiments. AAV-αCaMKII mice were fed with doxycycline solution (1 g/L in drinking water) to induce virus expression throughout the behavior tests.

2.4. Dendritic spine analysis

Dendritic spine analysis was performed as previously described (Ming et al., 2018). Briefly, mice were deeply anesthetized and transcardially perfused. Coronal brain sections (200 μm) were cut and collected in 0.1 M PBS. LA neurons were loaded iontophoretically with 5% Lucifer Yellow solution. Images of basal and apical dendrites of LA pyramidal neurons were scanned using a Leica SP2 confocal microscope at 63 × under oil immersion. The number of spines per micrometer along the dendritic longitudinal axis was counted as spine density. Data were presented as mean ± s.e.m. Mann Whitney test was used for statistical analysis.

2.5. Amygdala slice electrophysiology

Protocols were performed as described previously (Kim et al., 2007; Ma et al., 2013). Mice (3–4 months old) were anesthetized with sodium pentobarbital and sacrificed by decapitation. Whole brain coronal slices (370 μm thick for fEPSPs recording) containing the amygdala were cut using a vibroslicer (vibratome 3000) with cold (4 °C) modified artificial cerebrospinal fluid (ACSF) containing (in mM): Choline chloride, 110; KCl, 2.5; CaCl2, 0.5; MgSO4, 7; NaHCO3, 25; NaH2PO4, 1.25; D-glucose, 25; pH 7.4. The slices were recovered in an incubation chamber with normal ACSF containing (in mM): NaCl, 119; CaCl2, 2.5; KCl, 2.5; MgSO4, 1.3; NaHCO3, 26.2; Na2HPO4, 1.0; D-glucose, 11, pH 7.4; 95% O2 and 5% CO2 for 60 min at 31 °C, and then returned to room temperature for at least 1 h before recording.

2.6. Field excitatory postsynaptic potential recording

A stimulating electrode was placed in the fibers from the internal capsule to activate the thalamic input to the lateral amygdala (T-LA) synapses. A recording electrode was positioned in the LA to record field excitatory postsynaptic potential (fEPSP). Test responses were elicited at 0.033 Hz. After obtaining a stable baseline response for at least 15 min, LTP or LTD was induced. LTP was induced by applying 2 trains of high frequency stimulation (100 Hz for 1 s) with 10 s interval or 3 trains theta burst stimulation (10 bursts delivered every 200 ms, each burst consisted of 4 pulses at 100 Hz) with 10 s interval. For LTD induction, the standard 1 Hz protocol (1 Hz for 15 min) and 3 Hz protocol (3 Hz for 5 min) were used. Depotentiation was induced by applying 2 trains of high frequency stimulation (100 Hz for 1 s) with 10 s interval followed by the standard 1 Hz protocol (1 Hz for 15 min) after 20 min. For chemical-LTD induction, NMDA (Sigma, 30 μM in ACSF) was infused into the slice chamber for 3 min. Data were presented as mean ± s.e.m. Mann Whitney test (for comparing two different groups with Gaussian distribution) and one-way ANOVA followed by HSD post-hoc test with Bonferroni’s correction (for comparing more than two different groups) were used for statistical analysis.

2.7. Protein sample preparation

Combined with the previous protocol (Cai et al., 2011; Yin et al., 2013), synaptosomes were prepared as follows. LA tissues were homogenized in 1.5 ml homogenate-buffer (320 mM sucrose, 5 mM HEPES, pH 7.4) containing freshly added PMSF, PIC and PIC3. Homogenates were centrifuged at 500 g for 5 min to yield insoluble components. Then the supernatant fraction was collected and centrifuged at 10,000 g for 10 min to yield precipitation. The precipitation pellet was resuspended in 2 ml of 0.32 M sucrose, layered onto 2.25 ml of 0.8 M sucrose, and centrifuged at 98,000 g for 15 min using a swinging bucket rotor. Synaptosomes were collected from the 0.8 M sucrose layer and concentrated by centrifugation at 20,800 g for 45 min. Then the precipitation was resuspended in synaptosome lysis buffer (30 mM Tris (pH 8.5), 5 mM magnesium acetate, 8 M Urea, and 4% W/V CHAPS). For total proteins preparation, the LA areas were homogenized with RIPA buffer containing freshly added PMSF, PIC, and PIC3 and lysed on ice for 30 min, centrifuged at 10,000 g at 4 °C for 5 min, and total protein samples were taken as supernatant. Then the protein samples were stored in a –80 °C freezer until used. Protein concentration was quantified by a Pierce BCA Protein Assay kit (Thermo Scientific) after which protein was stored at –20 °C.

2.8. Western blot

Each sample of protein (5 μg/lane) was separated by 10% SDS-PAGE (P40650, NCM Biotech) at 120 V for 120 min. Then the separated proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane. The PVDF membranes were blocked in solution (5% skim milk and 1% BSA) at room temperature for 1h. A reversible Ponceau S staining of the membranes was done to normalize the relative amount of each protein on the membrane (just for synaptosomes). After washing with TBST buffer, the PVDF membranes were immunoblotted with the following antibodies: GluA1 antibody (1:2,000, Santa Cruz), GluA2 antibody (1:2,000, Millipore), pGluA1-Ser845 antibody (1:500, Abcam), GluA1-Ser831 antibody (1:500, Abcam), oCaMKII antibody (1:3,000, Abcam), p-αCaMKII-Thr286 antibody (1:20,000, Santa Cruz), pCaMKII antibody (1:2,000, Invitrogen), β-actin antibody (1:20,000, Sigma), GAPDH antibody (1:20,000, Proteintech), synapsin (SYP) antibody (1:2,000, Proteintech), TIF antibody (1:2,000, Abcam), Tubulin antibody (1:1,000, Millipore) at 4 °C for 12h. After washing with TBST buffer, the blots were reacted with an HRP-conjugated secondary antibody at room temperature for 1 h. Band intensity on the blot was quantified by an ECL immunoblotting detection system (Bio-Rad). Data were shown as mean ± s.e.m. Statistical differences were analyzed using post hoc test with Bonferroni’s correction following one-way ANOVA.

2.9. PP2A activity measurement

PP2A activity was measured by using immunoprecipitation phosphatase assay kit according to the manufacturer’s instructions (Catalog # 17–313, Millipore). Statistical differences were analyzed using post hoc test with Bonferroni’s correction following one-way ANOVA. Data were shown as mean ± s.e.m.

2.10. PP2B activity measurement

The activity of calcineurin (PP2B) was assayed by using a calcineurin cellular activity assay kit (207007, Millipore) by following the manufacturer’s instructions. Statistical differences were analyzed using post hoc test with Bonferroni’s correction following one-way ANOVA. Data were shown as mean ± s.e.m.

2.11. Statistical analysis

Statistical significance was assessed by one-way ANOVA, two-way ANOVA analysis of variance, or Mann Whitney test, where appropriate. Significant effects in analysis of variances were followed up with Bonferroni post-hoc tests. Statistical differences were analyzed using post hoc test with Bonferroni’s correction following one-way ANOVA. Data were shown as mean ± s.e.m.
3. Results

3.1. PTSD susceptible mice exhibit increased αCaMKII and reduced AMPAR internalization in the LA

PTSD susceptible (PS) individuals were identified in the underwater-trauma (UWT)-exposed group (23 male mice) and 4-CS/US-exposed group (23 male mice) by employing the behavioral profiling approach as described in the MATERIALS AND METHODS section (Fig. 1A1A2). PTSD susceptible mice exhibited persistently higher levels of cued freezing responses through extinction trials (Fig. 1B, PS-UWT vs. Control, F(4, 85) = 6.33, P < 0.001; PS-4CS/US vs. Control, F(4, 85) = 4.70, P < 0.01), spent significantly less time in the center area of the open field (OF) chamber (Fig. 1C, PS-UWT vs. Control, P < 0.01; PS-4CS/US vs. Control, P < 0.001), in the light zone of the light/dark (LD) box test (Fig. 1D, PS-UWT vs. Control, P < 0.01; PS-4CS/US vs. Control, P < 0.001), and in the open arms of water O maze (WOM) test (Fig. 1E, PS-UWT vs. Control, P < 0.01; PS-4CS/US vs. Control, P < 0.001) compared with control mice. Behavioral profiling revealed that only 7 mice each group showed PTSD-like behaviors in 23 mice exposed to UWT or 23 mice exposed repeatedly to US/CS.

The LA is a key brain region for fear extinction and anxiety-like behaviors (Jacques et al., 2019; Erlich et al., 2012; Grosso et al., 2018; Kim et al., 2007; Krabbe et al. 2018; Mahan and Ressler 2012; Schafe et al. 2005; Forster et al., 2012; Ressler 2010). CaMKII plays an important role in memory extinction (Bevilaqua et al., 2006; Szapiro et al., 2003; Burgdorf et al., 2017). Moreover, GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization contribute to fear extinction (Dalton et al., 2008; Kim et al., 2007; Lin et al., 2010; Bai et al., 2014; Hollis et al., 2016; Talukdar et al., 2018; Lee et al., 2013). Thus, we investigated levels of αCaMKII, βCaMKII, GluA1-Ser845/Ser831 phosphorylation and synaptic GluA1/2 expression in the LA of PTSD susceptible mice, and found that αCaMKII and the phosphorylated (p)-αCaMKII at Thr286 (p-αCaMKII-Thr286) were significantly up-regulated in PTSD susceptible mice that experienced either UWT or 4-CS/US exposure (Fig. 1F, PS-UWT vs. Control, αCaMKII, P < 0.01; p-αCaMKII-Thr286, P < 0.05; PS-4CS/US vs. Control, αCaMKII, P < 0.05; p-αCaMKII-Thr286, P < 0.05). However, no significant difference was observed in βCaMKII among the three groups (Fig. 1F, P > 0.05). In addition, PTSD susceptible mice showed significantly higher synaptic expression levels of GluA1/2 and phosphorylated GluA1-Ser845/Ser831 (Fig. 1F, PS-UWT vs. Control, GluA1: P < 0.01, GluA2: P < 0.01, GluA1-Ser831: P < 0.01, GluA1-Ser845: P < 0.01; PS-4CS/US vs. Control, GluA1, P < 0.001, GluA2: P < 0.05, GluA1-Ser831: P < 0.01, GluA1-Ser845: P < 0.01). Taken together, these results suggest that PTSD susceptible mice display a significantly higher levels of αCaMKII and lower levels of GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization in the LA.

3.2. Increasing αCaMKII in the LA is sufficient to cause PTSD-like phenotypes in both αCaMKII-F89G TG and AAV-αCaMKII mice

To further investigate whether increased αCaMKII in the LA leads to PTSD-like phenotypes such as impaired fear extinction and anxiety-like behaviors, we temporally and spatially manipulated αCaMKII overexpression in rats by employing an inducible and reversible chemical-genetic technique described in MATERIALS AND METHODS section. The LA displayed the higher level of αCaMKII/p-αCaMKII-Thr286 (Supplemental information, Fig. 5A1B) and normal morphology in the LA (Supplemental information, Fig. 5C1D).

Then, cued fear memory recall and cued fear extinction were measured after only 1-CS/US for cued fear conditioning (Fig. 2A). Given that forebrain αCaMKII overexpression impairs fear memory retrieval in our previous study (Cao et al., 2008), to examine the effect of αCaMKII overexpression on cued fear extinction in mice, we designed the "normal αCaMKII levels during cued fear memory retrieval but elevated αCaMKII levels during cued fear extinction period" paradigm by a single i.p. injection of NM-PP1 into both TG mice and WT littermates 15 min before the first recall test of cued fear memory (Fig. 2A). Under this paradigm, TG mice exhibited normal retrieval of cued fear memory in comparison to that of wild-type littermate (Fig. 2B, TG + i.p. vs. WT + i.p., P > 0.05). However, during cued fear extinction trials, WT mice had a significantly decreased freezing behavior compared with TG mice (Fig. 2B, TG + i.p. vs. WT + i.p., F(3, 264) = 10.73, P < 0.001). A post hoc analysis revealed that TG mice exhibited significantly higher level of freezing response to the CS in cued fear extinction trial 2, 3 and 4 (Fig. 2B, TG + i.p. vs. WT + i.p., P < 0.05), suggesting that increased αCaMKII may impair cued fear extinction. In addition, TG mice spent significantly less time (Fig. 2C-E, TG + i.p. vs. WT + i.p., P < 0.001) in the center area of the OF chamber (Fig. 2C), in the light zone of the LD test (Fig. 2D), and in the open arms of the elevated plus maze (EPM) test (Fig. 2E) compared with WT mice. Together, these findings indicate that increased αCaMKII in the LA may cause PTSD-like phenotypes.

To further confirm whether PTSD-like phenotypes in TG mice are due to the overexpression of αCaMKII-F89G protein, we then designed the "normal αCaMKII level during both fear memory recall and extinction period" paradigm by i.p. injection of NM-PP1 15 min before recall test and oral (p.o.) administration throughout the entire fear extinction period (Fig. 2B). Under this paradigm, TG mice had a freezing response similar to WT mice during cued extinction trials (Fig. 2B, TG + i.p. + p.o. vs. WT + i.p., P > 0.05), suggesting impaired cued fear extinction was rescued by NM-PP1 treatment in TG mice. Moreover, NM-PP1 had no effect on cued fear extinction in WT mice (Fig. 2B, WT + i.p. + p.o. vs. WT + i.p., P > 0.05), excluding the possibility that the rescuing effects by NM-PP1 were due to ‘facilitating extinction’ effects. In addition, TG mice treated with NM-PP1 spent comparable amounts of time (Fig. 2C-E, TG + i.p. + p.o. vs. WT + i.p., P > 0.05) in the center area of the open field chamber (Fig. 2C), in the light box of the LD test (Fig. 2D) and in the EPM test (Fig. 2E) compared with WT mice. Furthermore, TG mice without any treatment exhibited normal locomotor activity, exploratory behavior, and pain threshold (Supplemental information, Fig. 52). Taken together, we conclude that increased αCaMKII is indeed sufficient to produce PTSD-like phenotypes including impaired fear extinction and anxiety-like behaviors.

To further examine whether increasing αCaMKII specifically in the LA is sufficient to cause PTSD-like phenotypes, we bilaterally injected viral vectors AAV-αCaMKII (pAAV-TRE-αCaMKII-P2A-EF1-GFP-CMV-rTA) into the LA of C57BL/6J mice to overexpress αCaMKII specifically in the LA (Fig. 3A). As expected, both αCaMKII and p-αCaMKII-Thr286 expression levels significantly increased in the LA of AAV-αCaMKII mice (Fig. 3GH, αCaMKII, P < 0.01; p-αCaMKII-Thr286, P < 0.01). We performed cued fear memory test 24 h after 1-CS/US pairing. AAV-αCaMKII mice exhibited impaired cued fear memory during recall test (Fig. 3C, P < 0.001), which is consistent with our previous finding that αCaMKII overexpression impairs retrieval of fear memory (Cao et al., 2008). In addition, AAV-αCaMKII mice showed significantly impaired fear extinction (Fig. 3C, AAV-αCaMKII vs. AAV-control, F(4, 81) = 2.63, P < 0.05). Post hoc analysis revealed that AAV-αCaMKII mice exhibited freezing responses significantly higher than AAV-control mice on the 4th extinction trial (P < 0.05). In addition to the deficits in cued fear extinction, AAV-αCaMKII mice showed anxiety-like behaviors (Fig. 3D-F, AAV-αCaMKII vs. AAV-control, P < 0.001). These data suggest that increased αCaMKII expression specifically in LA is also sufficient to result in PTSD-like behaviors.

3.3. Increasing αCaMKII in the LA impairs AMPAR internalization and GluA1-Ser845/Ser831 dephosphorylation after cued fear extinction in both αCaMKII-F89G TG and AAV-αCaMKII mice

We quantified the expression of synaptic AMPAR composition subunits (GluA1/2) and GluA1-Ser845/Ser831 phosphorylation in the LA
before/after cued fear conditioning and extinction trials. After cued fear extinction trials, compared with cued fear conditioning trial, significant decreases in the GluA1/2 synaptic expression and phosphorylated GluA1-Ser845/Ser831 levels were found only in WT mice (Fig. 2F, WT vs. Ext, GluA1: P < 0.001; GluA2: P < 0.01; pGluA1-Ser845: P < 0.05; pGluA1-Ser831: P < 0.01), but not in TG mice (TG + FC vs. TG + Ext, GluA1/A2, pGluA1-Ser845/Ser831: P > 0.05). Furthermore, the GluA1/2 synaptic expression and phosphorylated GluA1-Ser845/Ser831 in TG mice were significantly higher than those in WT mice after cued fear extinction trials (Fig. 2F, TG + Ext vs. WT + Ext, GluA1/A2: P < 0.01; pGluA1-Ser845/Ser831: P < 0.05). Consistently, the input-output curve observed was higher in TG than in WT mice after cued fear extinction trials (Fig. 2G, TG vs. WT, P < 0.001), which could be recovered by 0.5 μM NM-PP1 (Fig. 2G, TG + NM-PP1 vs. TG, P < 0.001; TG + NM-PP1 vs. WT, P > 0.05).

Moreover, consistent with the above western blotting data from CaMKII-F89G TG mice, synaptic GluA1/2 expression and phosphorylated GluA1-Ser845/Ser831 levels were significantly higher in the LA of AAV-CaMKII mice than those in AAV-control mice after cued fear extinction trials (Fig. 3G, GluA1/A2, pGluA1-Ser845/Ser831, P < 0.01). Taken together, these results indicate that increasing αCaMKII specifically in the LA disrupts GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization, and may consequently impair cued fear extinction in both αCaMKII-F89G TG and AAV-αCaMKII mice.

3.4. Increasing αCaMKII impairs NMDAR-dependent LTD at T-LA synapses and NM-PP1 can recover the impairments

To investigate the cellular mechanism of impaired cued fear extinction and anxiety-like behaviors, we measured the basal synaptic transmission and synaptic plasticity at T-LA synapses in TG mice (Dalton et al., 2012; Xu et al., 2019). No significant difference was observed in input-output curves, synaptic and total GluA1/2 expression of LA (Supplemental information, Fig. S3AB, TG vs. WT, P > 0.05), paired-pulse depression (PPD) and synaptic expression (Fig. S3CD, TG vs. WT, P > 0.05) in LA between TG and WT mice. Moreover, either tetanic or theta burst stimulations induced similar level of LTD at T-LA synapses in two groups (Fig. 4AB, TG vs. WT, P > 0.05). These results indicate that αCaMKII overexpression does not affect basal synaptic transmission and LTP at T-LA synapses.

We then analyzed the effects of αCaMKII overexpression on LTD at T-LA synapses. 1Hz-LTD in TG slices was significantly reduced compared to that of WT slices (Fig. 4C, TG vs. WT, P < 0.05), which could be recovered by 0.5 μM NM-PP1 (Fig. 4C, TG + NM-PP1 vs. TG, P < 0.05), while 1Hz-LTD in WT slices was not affected (Fig. 4C, WT + NM-PP1 vs. WT, P > 0.05). Notably, LTD at the T-LA synapses could be blocked by application of APV (50 μM) and NM-PP1 (0.5 μM) (Fig. 4D, TG vs. WT, P > 0.05), suggesting that the LTD at the T-LA synapses is NMDAR-dependent. Additionally, 3Hz-LTD was blocked in TG slices (Fig. 4E; TG vs. WT, P < 0.001), which could also be recovered by NM-PP1.

Fig. 4. Increasing αCaMKII impairs NMDAR-dependent LTD at T-LA synapses of αCaMKII-F89G TG mice and NM-PP1 rescues the impairments. (A) Similar LTP induced by high frequency stimulations (2 trains of 100 Hz stimulation for 1 s, 10 s interval) in TG slices and WT slices. In this figure and the subsequent ones, insets show sample traces taken at baseline (1) and the last 10 min recording (2). (B) Normal LTP induced by three trains of theta burst stimulations (TBS, each train consisted of 10 bursts delivered at 5 Hz, each burst consisted of 4 pulses at 100 Hz) in TG slices. (C) Significantly weaker LTD induced in TG slices than that in WT slice after 1 Hz (15 min) stimulation. NM-PP1 (0.5 μM) recovered the reduced LTD in TG slice to normal level. (D) LTD was abolished in WT and TG slices exposed to both NM-PP1 (0.5 μM) and APV (50 μM). The solid lines show the duration of both NM-PP1 (red) and APV (blue) application. (E) Strong LTD could be induced by 3 Hz (5 min) stimulation in WT slices but not in TG slices. NM-PP1 (0.5 μM) rescued the impaired LTD in TG slice. (F) Impaired depotentiation can be observed in TG slices. All bar graphs summarize data obtained during last 10 min recording. Statistical differences were evaluated with Mann-Whitney test (A, B, D and F) and one-way ANOVA followed by Bonferroni’s multiple comparisons test (C and E). n.s.: not significant, *P < 0.05, **P < 0.01, ***P < 0.001. All values are mean ± s.e.m. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Fig. 5. Increasing αCaMKII impairs AMPAR internalization/phosphorylation, reduces protein phosphatase (PP) activity, and increases stargazin expression during NMDAR-dependent LTD and NM-PP1 rescues all impairments. (A) Attenuated chem-LTD induced by NMDA (30 μM, 3 min) could elicit a significantly weaker LTD at T-LA synapses than that in WT slices (Fig. 5A, TG + NMDA vs. WT + NMDA, P < 0.01). Furthermore, 0.5 μM NM-PP1 could rescue the reduced NMDA-induced LTD in TG slices to normal level (Fig. 5A, TG + NMDA + NM-PP1 vs. WT + NMDA, P > 0.05; TG + NMDA + NM-PP1 vs. TG + NMDA, P < 0.01), but had no detectable effects on NMDA-induced LTD in WT slices (Fig. 5A, WT + NMDA + NM-PP1 vs. WT + NMDA, P > 0.05). These results suggest that increasing αCaMKII in the LA attenuates NMDAR-dependent chem-LTD at T-LA synapses in TG mice.

NM-PP1-induced LTD elicits more widespread depression of synapse strength and shares certain molecular mechanisms with LFS-LTD such as AMPAR internalization and GluA1-Ser845/Ser831 dephosphorylation (Delgado et al., 2007; He et al., 2011; Kollen et al. 2008; Lee et al., 1998). We found the synaptic expression levels of GluA1/2 and GluA1-Ser845/Ser831 phosphorylation in the LA of TG slices were significantly higher than that in LA of WT slices (Fig. 5B, TG + NMDA vs. WT + NMDA, GluA1: P < 0.05; GluA2: P < 0.01; pGluA1-Ser845/Ser831: P < 0.05), which is consistent with the higher input-output responses in TG mice after impaired cued fear extinction as shown in Fig. 2G. Furthermore, NM-PP1 (0.5 μM) successfully rescued the impairments of AMPAR internalization and GluA1-Ser845/Ser831 dephosphorylation of the LA in TG slices (Fig. 5B, TG + NMDA + NM-PP1 vs. TG + NMDA, GluA1/A2, pGluA1-Ser845/831: P < 0.01), with no effect on that of WT slices (Fig. 5B, WT + NMDA + NM-PP1 vs. WT + NMDA, GluA1/A2, pGluA1-Ser845/831: P > 0.05). Collectively, these findings indicate that αCaMKII overexpression leads to the impairment of AMPARs internalization and dephosphorylation in the LA, which consequently impairs NMDAR-dependent LTD at T-LA synapses.

Besides low-frequency stimulation (LFS), brief NMDA exposure can chemically induce NMDAR-dependent LTD (Lee et al., 1998). In TG slices, NMDA application (30 μM, 3 min) could elicit a significantly weaker LTD at T-LA synapses than that in WT slices (Fig. 5A, TG + NMDA vs. WT + NMDA, P < 0.01). Furthermore, 0.5 μM NM-PP1 could rescue the reduced NMDA-induced LTD in TG slices to normal level (Fig. 5A, TG + NMDA + NM-PP1 vs. WT + NMDA, P > 0.05; TG + NMDA + NM-PP1 vs. TG + NMDA, P < 0.01), but had no detectable effects on NMDA-induced LTD in WT slices (Fig. 5A, WT + NMDA + NM-PP1 vs. WT + NMDA, P > 0.05). These results suggest that increasing αCaMKII in the LA attenuates NMDAR-dependent chem-LTD at T-LA synapses in TG mice.
3.6. Increasing αCaMKII reduces protein phosphatase (PP) activity and enhances stargazin expression during NMDAR-dependent LTD and NM-PP1 can recover these abnormalities

Activation of protein phosphatase 1 (PP1) contributes to LTD formation (Mansuy and Shenolikar 2006; Mauna et al., 2011). Moreover, stargazin can be dephosphorylated by PP1 to induce the clathrin-dependent AMPAR endocytosis during NMDAR-dependent LTD (Matsuda et al., 2013; Bats et al. 2007). Dephosphorylation of the Thr320 residue on the C-terminal domain of PP1 can enhance PP1 activity during NMDAR-dependent LTD (Dohadwala et al., 1994; Goldberg et al., 1995). Therefore, we investigated PP1-Thr320 phosphorylation (ppPP1-Thr320) and stargazin expression in the LA fractions of WT and TG slices with NMDA treatment. With NMDA exposure, the PP1-Thr320 phosphorylation and stargazin expression in LA of TG slices were dramatically higher than that in WT slices (Fig. 5DE, TG vs. WT + NMDA, pPP1-Thr320, stargazing, P < 0.05), suggesting that the PP1 activity and stargazin expression were abnormal in TG mice during NMDA-induced LTD. Furthermore, NM-PP1 could recover the abnormalities in PP1 activity and stargazin expression in the LA of TG slices (Fig. 5DE, TG + NMDA + NM-PP1 vs. TG, pPP1-Thr320: P < 0.01; stargazing: P < 0.01; TG + NMDA + NM-PP1 vs. TG + NMDA, pPP1-Thr320: P < 0.05, stargazing: P < 0.05) but did not affect their levels in WT slices (Fig. 5DE, WT + NMDA + NM-PP1 vs. WT + NMDA, pPP1-Thr320: P > 0.05; stargazing: P > 0.05). Therefore, we investigated PP1-Thr320 phosphorylation (ppPP1-Thr320) and stargazin expression in the LA fractions of WT and TG mice during NMDA-induced LTD, which may cause the impairments in AMPAR internalization and NMDAR-dependent LTD.

4. Discussion

In the present study, we reveal that PTSD susceptible mice exhibit significant up-regulation of αCaMKII, down-regulation of GluA1-Ser845/Ser831 dephosphorylation, and reduction in AMPAR internalization in LA. Consistently, increasing αCaMKII specifically in the LA leads to PTSD-like phenotypes such as fear extinction deficit and anxiety-like behaviors, and impairs AMPAR internalization and dephosphorylation, NMDAR-dependent LTD and depotentiation at T-LA synapses. Furthermore, deficits in AMPAR internalization and dephosphorylation are observed not only after impaired cued fear extinction in vivo, but also after attenuated NMDA-induced LTD in TG slices in vitro. Additionally, the deficits in AMPAR internalization and dephosphorylation are due to down-regulation of PP1/2A, PP2B activity and increased stargazin in TG mice. Importantly, NM-PP1, a specific inhibitor of the exogenous αCaMKII-F89G, could rescue the above deficits in αCaMKII-F89G TG mice. These results identify αCaMKII as a potential molecular determinant of PTSD-like behaviors and a powerful regulator of LTD at T-LA synapses.

4.1. αCaMKII and fear extinction

Previous studies have implicated changes as in CaMKII related to extinction of different memories. Specifically, pharmacological inhibition of CaMKII by KN-62 blocked the extinction of step-down passive avoidance performance (Bevilaqua et al., 2006; Szapiro et al., 2003). Similarly, αβCaMKII inhibitor KN93 significantly attenuated the extinction of cocaine conditioned place preference (Burgdorf et al., 2017). Furthermore, partial reduction of αCaMKII function due to T286A mut mutation impaired the extinction of contextual fear and spatial memories (Kimura et al. 2008). Paradoxes also exist, for example, reduction of αCaMKII by phosphorylation at serine 331 in the LA enhances cocaine memory extinction (Rich et al., 2016). Furthermore, increased activation of αCaMKII in the CPEB3-knockout hippocampus reduced the extinction of spatial memories (Berger-Sweeney et al., 2006; Huang et al., 2014). These conflicting findings implied the controversial roles of CaMKII in extinction of different memories. Thus, whether and which CaMKII sub-type are necessary or sufficient for fear extinction remains to be determined. In our study, we found that mouse models of PTSD with cued fear extinction deficit exhibited significant up-regulation of αCaMKII in the LA. Consistently, increasing αCaMKII in the LA impaired cued fear extinction. Furthermore, returning the increased αCaMKII to normal level could completely restore the impaired fear extinction. Our results thus indicate that αCaMKII may serve as a potential molecular determinant of fear extinction.

4.2. αCaMKII and LTD

Endogenous CaMKII plays pivotal roles in synaptic plasticity (Collingridge et al. 2004). Specifically, CaMKII can promote either LTP or LTD (Pi et al., 2010). CaMKII can enhance LTP through promoting the integration of new AMPA receptors at the postsynaptic density (Barria et al., 1997). In our previous studies, αCaMKII up-regulation in the forebrain disrupted LTD in the medial prefrontal cortex (Ma et al., 2015). Similarly, βCaMKII up-regulation blocked LTD in the dentate gyrus (Yin et al., 2017). However, whether αCaMKII overexpression in the LA affects synaptic plasticity at T-LA synapses is unclear. Here we demonstrated that αCaMKII overexpression in the LA did not affect LTP but caused impairments in LTD at T-LA synapses in TG mice, which could be completely rescued by a specific inhibitor (NM-PP1) of exogenous αCaMKII-F89G. We conclude that αCaMKII may be a regulator of LTD at T-LA synapses.

4.3. The molecular links between LTD and fear extinction, and between LTD and anxiety-like behaviors

Previous studies indicated potential links between LTD and fear extinction. Optogenetic delivery of LTD stimuli to the auditory input to the LA facilitates cued fear extinction (Nabavi et al., 2014). Moreover, both hippocampal LTD and fear extinction were enhanced by aquaporin-4 deficiency (Wu et al., 2017). Furthermore, both hippocampal LTD and contextual fear extinction were impaired by kinesin superfamily proteins 21B (Morikawa et al., 2018). Similarly, both LTD at T-LA synapses and fear extinction could be blocked by GluN2B receptor antagonist (Dalton et al., 2012). In addition to the relation between LTD and fear extinction, accumulated evidences also indicated the relation between LTD and anxiety-like behaviors (Zhang et al., 2017; Xu et al., 2019). However, it remains unclear what molecules links between LTD and fear extinction, and between LTD and anxiety-like behaviors are unclear. GluA1-Ser845/Ser831 dephosphorylation (Hollis et al., 2016) and AMPAR internalization (Dalton et al., 2008; Bai et al., 2014) have been implicated in the regulation of fear extinction. Moreover, previous studies have shown that GluA1-Ser845 dephosphorylation and AMPAR internalization are crucial for LTD expression (Lee et al., 2005; Carroll et al., 2001). Although these findings indicate GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization may be a potential link between fear extinction and LTD, a direct evidence remains to be elucidated. In our current study, the deficits in GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization were observed not only after impaired cued fear extinction and increased anxiety-like behaviors in vivo, but also after attenuated NMDA-induced LTD in αCaMKII-F89G TG slices in vitro. Furthermore, a specific inhibitor of the exogenous...
αCaMKII-F89G (NM-PP1) could completely rescue not only the increased anxiety-like behaviors and deficits in cued fear extinction, but also NMDA-induced LTD, GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization. Notably, the input-output responses in TG slices were significantly higher than WT slices after cued fear extinction and increased anxiety-like behaviors, which could also be reversed by NM-PP1. Besides αCaMKII-F89G TG mice, PTSD susceptible mice and AAV-αCaMKII mice displayed a significantly higher level of αCaMKII, lower levels of GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization in the LA. These mice also exhibited the deficits in cued fear extinction and showed anxiety-like behaviors. Based on the molecular and behavioral characteristics, we hypothesize that PTSD susceptible mice and AAV-αCaMKII mice might also exhibit impairments in LTD at t-LA synapses. Therefore, further studies that examine the LTD in the LA of these mice are required to fully comprehend increased αCaMKII of different mice in mediating synaptic plasticity.

Taken together, these data demonstrate that deficits in Ser845/GluA1-Ser831 dephosphorylation and AMPAR internalization by increased αCaMKII are molecular links not only between impaired NMDAR dependent-LTD and fear extinction, but also between impaired LTD and increased anxiety-like behaviors.

4.4. How does increased αCaMKII impair AMPAR internalisation and dephosphorylation during NMDAR-dependent LTD?

CaMKII is the major kinase mediating NMDAR-dependent synaptic plasticity and AMPAR trafficking (Collingridge et al., 2004). In mammals, CaMKII has four isoforms, α, β, γ, and δ (Colbran and Soderling 1990; Hell 2014), and the α isoform is predominantly expressed in the forebrain (Kennedy et al. 1983). LTD formation requires PPs (PP1, PP2A and PP2B) activation (Kameyama et al., 1998; Lee et al., 1998). Activated PPs dephosphorylate GluA1-Ser845/Ser831 (Hu et al., 2007; Winder and Sweatt 2001; Mansuy and Shenolikar 2006), which cause a reduction in the probability of AMPAR channels being open or their conductance, and finally contribute to LTD formation. Specifically, PP1 is activated through a Ca\(^{2+}\)-PP2B-I1 pathway and has a more predominant role in depressing potentiated synapses, whereas PP2A is activated through PP2B/PP1 cascade or pathways independent on PP2B and mainly depresses naïve synapses (Winder and Sweatt 2001). However, in αCaMKII-F89G TG mice, αCaMKII overexpression could exhibit higher potency in the competition with PP2B for Ca\(^{2+}\)/CaM, which might decrease the accessibility of PP2B to Ca\(^{2+}\)/CaM and inhibit the activity of the PP2B-I1-PP1 pathway, thereby inhibiting PP1 activity. In addition, high concentration of phosphorylated αCaMKII could saturate the dephosphorylation ability of PP1, and thereby weaken PP1 dephosphorylating GluA1-Ser845 or GluA1-Ser831 (Lisman and Zhabotinsky 2001; Lee et al., 2000; Hu et al., 2007; Winder and Sweatt 2001; Mansuy and Shenolikar 2006). Unlike PP1, PP2A can be directly inactivated by CaMKII through phosphorylating its B\(^-\)α subunits (Fukunaga et al., 2000; Pi and Lisman 2008), therefore, excessive CaMKII can weaken PP2A activity. Collectively, one explanation for impairment of NMDAR-dependent LTD is that the excessive αCaMKII can reduce the activity of PPs, thereby reducing GluA1-Ser845/Ser831 dephosphorylation and AMPAR internalization, and consequently impairing LTD. It has been shown that stargazin can be dephosphorylated by PP1 through Ca\(^{2+}\)-PP2B-II pathway and form a ternary complex with APs to promote AMPAR internalization during NMDAR-dependent LTD (Matsumura et al., 2013; Tomita et al., 2003). Conversely, stargazin can be directly phosphorylated by activated CaMKII and bind to PSD-95 to immobilize AMPARs at synapses, which contributes to LTD (Bats et al., 2007; Opazo et al., 2010). In αCaMKII-F89G TG mice, more stargazin is expressed at the synaptic sites during NMDAR-dependent LTD. Therefore, another explanation for impairment of NMDAR-dependent LTD is that excessive CaMKII weakens AMPAR internalization through directly increasing stargazin phosphorylation and indirectly reducing stargazin dephosphorylation caused by lower PP1 activity, which finally impairs LTD.

Funding

This research was supported by National Natural Science Foundation of China (No: 31771177 and 31471077 to Xiaohua Cao, 31970944 to Shuming An) and fund from MOST China-Israel cooperation (No: 2016YFE0130500 to Xiaohua Cao).

CRediT authorship contribution statement

Shuming An: Formal analysis, Data curation, Visualization, Writing – original draft, Drafting – review & editing, Conceptualization, Resources, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition, Supervision. Jiayue Wang: Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. Xuliang Zhang: Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. Yanhong Duan: Formal analysis, Software, Validation. Yiqiong Xu: Formal analysis, Software, Validation. Junyan Lv: Formal analysis, Software, Validation. Dasheng Wang: Formal analysis, Software, Validation. Gal Richter-Levin: Writing – review & editing. Oded Klavir: Writing – review & editing. Buwei Yu: Conceptualization, Resources, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition, Supervision. Xiaohua Cao: Conceptualization, Resources, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We thank Dr. Xuechu Zhen and Dr. Liyong Li for their assistance with immunoblotting study, Dr. Kevan M. Shokat of UCSF for providing NM-PP1 inhibitor, and Dr. Bo Wang, Dr. Yihui Cui for their comments on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2021.100359.

References

Ardi, Z., Albrecht, A., Richter-Levin, A., Saha, R., Richter-Levin, G., 2016. Behavioral profiling as a translational approach in an animal model of posttraumatic stress disorder. Neurobiol. Dis. 88, 139–147.
Bai, Yanrui, Zhou, Limin, Wu, Xiaoyan, Dong, Zhihong, 2014. d-Serine enhances fear extinction by increasing GluA2-containing AMPA receptor endocytosis. Behav. Brain Res. 270, 223–227.
Barria, A., Muller, D., Derkach, V., Griffith, L.C., Soderling, T.R., 1997. Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-kinase II during long-term potentiation. Science 276, 2042–2045.
Bats, Cecile, Grog, Laurent, Choquet, Daniel, 2007. The interaction between stargazin and PSD-95 regulates AMPA receptor surface trafficking. Neurosci 53, 719-734.
Bennett, M.R., Arnold, J., Hatton, S.N., Lagopoulou, J., 2017. Regulation of fear extinction by long-term depression: the roles of endocannabinoids and brain derived neurotrophic factor. Behav. Brain Res. 319, 148-164.
Berger-Sweeney, Joanne, Zearfoss, N., Richter, Joel, 2006. Reduced extinction of hippocampal-dependent memories in OPEB knockout mice. Learn. Mem. 13, 4–7.
Besv quia, I.R., Bonini, J.S., Rosato, J.I., Izquierdo, L.A., Izquierdo, I., 2016. The entorhinal cortex plays a role in extinction. Neurobiol. Learn. Mem. 85, 192–197.
Borghans, B., Homberg, J.R., 2015. Animal models for posttraumatic stress disorder: an overview of what is used in research. World J. Psychiatr. 5, 387–396.
Brener, K., Wong, T.P., Liu, L., Liu, Y., Campbell, P., Gray, S., Phelps, L., Phillips, A.G., Wang, Y.T., 2005. Nucleus accumbens long-term depression and the expression of hippocampal cAMP response element sequence. Science 310, 1340–1343.

Burgdorf, C.E., Schierberger, K.C., Lee, A.S., Fischer-Dempke, D.K., Van Kampen, T.A., Mudrigel, V., Huganir, R.L., Milner, T.A., Glass, M.J., Rajadhyaksha, A.M., 2017. Extinction of contextual cocaine memories requires Ca(v)1.2 within DLR-expressing cells and resistant hippocampal Ca(v)1.2-dependent signaling mechanisms. J. Neurosci. 37, 11894–11911.

Cao, X., Wang, H., Mei, B., An, S., Yin, L., Wang, L.P., Tsien, J.Z., Joo, J.S., 2008. Inducible and selective erasure of memories in the mouse brain via chemical-genetic manipulation. Neuron 60, 353–366.

Carroll, R.C., Beattie, E.C., von Zastrow, M., Malenka, R.C., 2001. Role of AMPA receptor endocytosis in LTD induction. Neuron 30, 367–375.

Carroll, R.C., Beattie, E.C., von Zastrow, M., Malenka, R.C., 2001. Role of AMPA receptor endocytosis in LTD induction. Neuron 30, 367–375.

Cui, Y., Jin, J., Zhang, X., Xu, H., Yang, L., Du, D., Zeng, Q., Tsien, J.Z., Yu, H., Cao, X., Collingridge, G.L., Isaac, J.T., Wang, Y.T., 2004. Receptor trafficking and synaptic function. Nat. Rev. Neurosci. 5, 535–543.

Colbran, R.J., Soderling, T.R., 1990. Calcium/calmodulin-dependent protein kinase II. Annu. Rev. Biochem. 59, 589–640.

Dalton, G.L., Wu, D.C., Wang, Y.T., Floresco, S.B., Phillips, A.G., 2008. Inducible and NMDA receptor-dependent Ca(v)2.2 receptor subunit interactions in the induction of LTD in the amygdala and in the acquisition and extinction of conditioned fear. Neuropharmacology 62, 797–806.

Davis, M., 1992. The role of the amygdala in fear and anxiety. Annu. Rev. Neurosci. 15, 251–284.

Deisseroth, K., 1998. Optogenetics in the brain. Annu. Rev. Neurosci. 21, 317–343.

Dell, 2007. NMDA receptor activation dephosphorylates AMPA receptor GluRa1 and GluRb2 receptor subunits in the induction of LTD and LTD in the amygdala and in the acquisition and extinction of conditioned fear. J. Neurochem. 82, 1320–1323.

Dobadwala, M., da Cruz e Silva, E.F., Hall, F.L., Williams, R.T., Carbonaro-Hall, D.A., Nairn, A.C., Greengard, P., Berndt, N., 1994. Phosphorylation and inactivation of protein phosphatase 1 by cyclin-dependent kinases. Proc. Natl. Acad. Sci. U. S. A. 91, 6408–6412.

Duan, Y., Zhou, S., Ma, J., Yin, P., Cao, X., 2015. Forebrain NR2B overexpression enhances fear acquisition and long-term potentiation in the lateral amygdala. Eur. J. Neurosci. 42, 2214–2230.

Etkin, Amit, Wager, Tor, 2007. Functional neuroimaging of anxiety: a meta-analysis of probabilistic reinforcement. Front. Behav. Neurosci. 6, 16–16.

Etkin, A., Aziz, N., Wager, T., 2007. Neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am. J. Psychiatry 164, 1476–1488.

Fanello, M.S., Gale, G.D., 2003. The amygdala, fear and memory. Ann. N. Y. Acad. Sci. 985, 125–134.

Fenster, R., Brown, K.M., Lebois, M., Fugger, R., Resler, S., Junghen, B., 2018. Brain circuit dysfunction in post-traumatic stress disorder from mouse to man. Nat. Rev. Neurosci. 19, 535–551.

Forster, G., Andrew, M., Novick, J., Scholl, M., 2012. The role of the amygdala in the retrieval and maintenance of fear memories formed by repeated probabilistic reinforcement. Front. Behav. Neurosci. 6, 16–16.

Forsythe, G., Mansuy, I.M., Shenolikar, S., 2006. Protein serine/threonine phosphatases in neuronal molecular regulation. Nat. Rev. Neurosci. 7, 753–764.

Forsythe, G., Mansuy, I.M., Shenolikar, S., 2006. Protein serine/threonine phosphatases in neuronal molecular regulation. Nat. Rev. Neurosci. 7, 753–764.

Forsythe, G., Mansuy, I.M., Shenolikar, S., 2006. Protein serine/threonine phosphatases in neuronal molecular regulation. Nat. Rev. Neurosci. 7, 753–764.

Fu, X., Xiang, Hai, Luo, Jia, Chen, Zhou, Huang, Xiao, Yuan, Xiaomeng, 2020. Lycopene ameliorates PTSD-like behaviors in mice and rebalances the neuroinflammatory response and oxidative stress in the brain. Physiol. Behav. 224, 113026.

Fukunaga, K., Muller, Dominique, Ohmitu, Masao, Bako, Eva, Anna, A., DeFau, Robin, Miyamoto, Noriko, 2000. Decreased protein expression due 2A activity in hippocampal long-term potentiation. J. Neuroscience 74, 807–817.

Goldberg, J., Huang, H.B., Kwon, Y.G., Greengard, P., Nairn, A.C., Kuryian, J., 1995. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1A. Nature 376, 745–755.

Grosso, Anna, Santoni, Giulia, Manassero, Eugenio, Renna, Annamaria, Benedetto Sacchetti, 2018. A neuronal basis for fear discrimination in the lateral amygdala. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1A. Nature 376, 745–755.
and physiological responses in a civilian population with high levels of trauma. Am. J. Psychiatr. 172, 353-362.

Milad, M.R., Quirk, G.J., 2012. Fear extinction as a model for translational neuroscience: ten years of progress. Annu. Rev. Psychol. 63 (63), 129–151.

Milad, Mohammed R., Quirk, Gregory J., 2011. Fear extinction as a model for translational neuroscience: ten years of progress. Annu. Rev. Psychol. 63, 129–151.

Ming, Chen, Shao, Da, Fa, Yali, Ma, Qianjuan, Cui, Dongyang, Song, Xiaojiao, Sheng, Huan, Yang, Li, Dong, Yi, Lai, Bin, Zheng, Ping. 2018. Key determinants for morphine withdrawal conditioned context-induced increase in Arc expression in anterior cingulate cortex and withdrawal memory retrieval. Exp. Neurol. 311.

Merey, Rajendra A., Emily, K., Clarke, Courtney, C., Haswell, Rachel D., Phillips, Ashley, N., Clausen, Mary, S. Mufford, Saygin, Zeynep, Brancu, Mira, Beckham, Jean C., Calhoun, Patrick S., Eric Deredt, Elbogom, Eric B., Fairbank, John A., Hurley, Robin A., Kils, Janson D., Kimbel, Nathan A., Kirby, Angela, Christine E., Marx, Scott D., McDonald, Scott D., Moore, Jennifer C., Naylor, Rowland, Jared, Swinkels, Cindy, Szabo, Steven T., Taber, Katherine H., Taper, Larry A., van Voorhees, Elizabeth E., Yoash-Gantz, Ruth E., Ryan Wagner, H., Lalbar, Kevin S., 2020. Amygdala nuclei volume and shape in military veterans with posttraumatic stress disorder. Biol. Psychiatr.: Cognit. Neurosci. Neuroimag. 5, 281–290.

Morikawa, M., Tanaka, Y., Cho, H.S., Yoshihara, M., Hirokawa, N., 2018. The molecular motor KIF21B Mediates synaptic plasticity and fear extinction by terminating Rac activation. Cell Rep. 23, 3864–3877.

Nabavi, S., Fox, R., Proulx, C.D., Lin, J.Y., Tsien, R.Y., Malinow, R., 2014. MPTP-Induced apoptosis of amygdala nuclei in traumatized youths. Transl. Psychiatry 10, 288.

Neylan, Thomas C., Hyman, Steven E., 2015. Post-traumatic stress disorder. Nat. Rev. Neurosci. 2, 461.

Nicoll, Roger A., Bredt, David S., 2003. Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. J. Cell Biol. 161, 805–816.

Ni, Nan, Zhang, T., Chen, T., Chen, P., Zhang, B., Hong, J., Chen, L., 2017. Protective effects of silibinin and its possible mechanism of action in mice exposed to chronic unpredictable mild stress. Biomed. Therapeut. 23, 245–250.

Ng, Yehuda, Rachel, Hoge, Charles W., McFarlane, Alexander C., Vermetten, Eric, Lane, Lisa, S. Mufford, Saygin, Zeynep, Brancu, Mira, Beckham, Jean C., Zhang, C., Morimoto, H., Zhuo, M., Feng, R., Shokat, K.M., Tien, J.Z., 2003. Inducible protein knockout reveals temporal requirement of CaMKII reactivation for memory consolidation in the brain. Proc. Natl. Acad. Sci. U. S. A. 100, 4287–4292.

Neylan, Thomas C., Hyman, Steven E., 2015. Post-traumatic stress disorder. Nat. Rev. Neurosci. 2, 461.

Nicoll, Roger A., Bredt, David S., 2003. Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. J. Cell Biol. 161, 805–816.

Neylan, Thomas C., Hyman, Steven E., 2015. Post-traumatic stress disorder. Nat. Rev. Neurosci. 2, 461.

Nicoll, Roger A., Bredt, David S., 2003. Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. J. Cell Biol. 161, 805–816.

Neylan, Thomas C., Hyman, Steven E., 2015. Post-traumatic stress disorder. Nat. Rev. Neurosci. 2, 461.

Nicoll, Roger A., Bredt, David S., 2003. Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. J. Cell Biol. 161, 805–816.