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A-Kinase Anchoring Protein 150 and Protein Kinase A Complex in the Basolateral Amygdala Contributes to Depressive-like Behaviors Induced by Chronic Restraint Stress

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ABSTRACT

BACKGROUND: The basolateral amygdala (BLA) has been widely implicated in the pathophysiology of major depressive disorder. A-kinase anchoring protein 150 (AKAP150) directs kinases and phosphatases to synaptic glutamate receptors, controlling synaptic transmission and plasticity. However, the role of the AKAP150 in the BLA in major depressive disorder remains poorly understood.

METHODS: Depressive-like behaviors in C57BL/6J mice were developed by chronic restraint stress (CRS). Mice received either intra-BLA injection of lentivirus-expressing *Akap5* short hairpin RNA or Ht-31, a peptide to disrupt the interaction of AKAP150 and protein kinase A (PKA), followed by depressive-like behavioral tests. Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptor (AMPAR)–mediated miniature excitatory postsynaptic currents were recorded by whole-cell patch-clamp techniques.

RESULTS: Chronic stress exposure induced depressive-like behaviors, which were accompanied by an increase in total and synaptic AKAP150 expression in the BLA. Accordingly, CRS facilitated the association of AKAP150 with PKA, but not of calcineurin in the BLA. Intra-BLA infusion of lentivirus-expressing *Akap5* short hairpin RNA or Ht-31 prevented depressive-like behaviors and normalized phosphorylation of serine 845 and surface expression of AMPAR subunit 1 (GluA1) in the BLA of CRS mice. Finally, blockage of AKAP150-PKA complex signaling rescued the changes in AMPAR-mediated miniature excitatory postsynaptic currents in depressive-like mice.

CONCLUSIONS: These results suggest that AKAP150-PKA directly modulates BLA neuronal synaptic strength, and that AKAP150-PKA-GluA1 streamline signaling complex is responsible for CRS-induced disruption of synaptic AMPAR-mediated transmission and depressive-like behaviors in mice.

Keywords: A-kinase anchoring protein 150, AMPA receptor, Basolateral amygdala, Chronic restraint stress, Depressive-like behaviors, Protein kinase A

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Major depressive disorder impacts approximately 15% of the population (1), and roughly one half of patients do not fully respond to available treatments (2). The neurobiology underlying depression has not yet been fully identified but is thought to result, at least in part, from the adaptive and maladaptive consequences of the basolateral amygdala (BLA) during stress (3,4). Under conditions of severe and repeated stress, disruption of synaptic transmission leads to more persistent changes in the synaptic plasticity (5). Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) mediates most of the fast excitatory synaptic transmission, which is known to be critical for glutamatergic synaptic plasticity and stress response (6,7). Accumulating evidence has linked glutamatergic system dysfunction to the pathophysiology of depression. In particular, ketamine and traxoprodil, *N*-methyl-D-aspartate receptor antagonists, produce an antidepressant effect (8–10), indicating that the ionotropic glutamate receptors may be the potential target for antidepressant treatment. Furthermore, preclinical studies have demonstrated that mouse strains present various anxiety- and depression-related responses to chronic stress in parallel with changes in glutamatergic signaling in the BLA (11).

Glutamatergic signaling, especially the activity of AMPARs, has been reported to be controlled by various regulatory proteins, and A-kinase anchoring proteins (AKAPs) signaling complexes have emerged as important regulators of AMPARs. AKAP5 (also called AKAP79 in humans or AKAP150 in rodents, AKAP79/150) is the best-characterized isoform of AKAP family scaffold proteins in the brain and is now recognized for its ability to form multiple protein complexes in different

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subcellular regions within nanodomain signalosomes. As a prominent synapse-target AKAP, AKAP79/150 links protein kinase A (PKA), protein kinase C (PKC), and the Ca^{2+}-dependent protein phosphatase calcineurin (CaN; also known as protein phosphatase 2B) with AMPARs. In particular, AKAP79/150 anchors both PKA and CaN, potentially poising the two enzymes to act antagonistically against each other to control AMPAR synaptic strength (12). AKAP150 directs PKA to AMPARs via interaction with the scaffolding protein, synaptic membrane-associated guanylate kinase, promoting phosphorylation of AMPAR subunit 1 (GluA1) at serine 845 (Ser845) residues and anchors CaN through a PxIxIT-type docking motif, responsible for the dephosphorylation (13,14). AKAP150-PKA–dependent phosphorylation leads to an increase in surface expression of GluA1-containing AMPARs, as well as the open probability of synaptic AMPARs (15–17), while AKAP150-anchored CaN opposes PKA phosphorylation of GluA1 to restrict synaptic incorporation of AMPARs (18). Evidently, the AKAP150 complex signaling system is critical in excitatory synaptic transmission and plasticity, learning, and stress processing (12,19–21).

Recent evidence shows aberrations in the *AKAP5* gene and changes in AKAP5 protein expression in individuals with mood disorder diseases (22,23). Thus, one of the possible mechanisms underlying stress-induced changes in glutamatergic signaling may be an unbalance of AKAP150-anchored kinases or phosphatases, leading to abnormal phosphorylation of AMPARs, especially GluA1. Internal and external stress exposure induces rapid neuronal activation in the BLA through stimulation of the cyclic adenosine monophosphate (cAMP)–dependent protein kinase pathway (24), and elevated intracellular levels of cAMP ultimately activate PKA. Therefore, we asked whether the AKAP150-PKA signaling complex in the BLA contributed to chronic stress–induced depressive-like behaviors.

In the present study, we investigated the role of AKAP150-PKA complex in AMPAR-mediated excitatory synaptic transmission in the BLA under chronic restraint stress (CRS). It was found that increased AKAP150-anchored PKA contributed to depressive-like behaviors via promoting AMPAR-mediated excitatory synaptic transmission. This finding will provide a novel therapeutic target for depressive disorders.

METHODS AND MATERIALS

Animals

C57BL/6J mice (6 weeks of age) were housed individually in home cages with a 12-hour light/dark cycle and with food and water ad libitum. The research complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals and was approved by the Animal Welfare Committee of Huazhong University of Science and Technology.

Restraint Stress Procedures

Mice were subjected to restraint stress after acclimated to the facility for 1 week. Restraint stress was performed based on a previously described procedure with minor modifications (25,26). Briefly, mice were restrained in 50-mL adjustable cylindrical plastic restrainers, fitted to allow them to breathe. For acute restraint stress (ARS), mice were immobilized in the tube once for 2 hours, whereas for chronic restraint stress, they were immobilized for 2 hours once daily for 7 consecutive days (CRS 7), 14 consecutive days (CRS 14), and 21 consecutive days (CRS 21). Mice assigned to nonstressed groups were untouched in their home cages. Behavioral assays were performed blindly without knowledge of the treatment history of the animal.

Behavioral Assessments

After stress, mice were subjected to behavioral tests, including the sucrose preference test (SPT), the tail suspension test (TST), the forced swim test (FST), and the open field test. The details are provided in the Supplemental Methods and Materials.

Statistical Analysis

All data were presented as the mean ± SEM. All statistics were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL). Comparison between two groups was evaluated by unpaired Student's *t* test. Differences in different treatment groups were carried out using one-way or two-way analysis of variance with Bonferroni's post hoc multiple comparison tests. Statistical significance was defined as $p < .05$. Full details are available in Supplemental Methods and Materials.

RESULTS

Chronic Stress Increases the Total and Synaptic AKAP150 in the BLA of Mice

Male mice were immobilized in the restrainer once for 2 hours (ARS procedure) or 2 hours once daily for 7, 14, and 21 consecutive days (CRS 7, CRS 14, and CRS 21 procedure). At the end of stress, depressive-like behaviors were assessed with the SPT, TST, and FST (Figure 1A). Compared with control animals, mice that underwent the CRS 14 and CRS 21 procedures, but not the ARS or CRS 7 procedure, displayed decreased sucrose preference in the SPT (Figure 1B). Pie charts also showed a relatively higher number of mice with preference deficits (sucrose consumption <0.75) (Supplemental Figure S1A). Moreover, the CRS 14 and CRS 21 procedures increased mice immobility time in the TST (Figure 1C) as well as floating time in the FST (Figure 1D). Accordingly, the numbers of mice with immobility time longer than average of control animals in the TST (90 seconds) and FST (85 seconds) were higher in the CRS 14 and CRS 21 groups (Supplemental Figure S1B, C). Notably, both ARS and CRS induced anxiety behaviors (Supplemental Figure S2) without changing the spontaneous activity of mice in the open field test (Figure 1E). Taken together, the mice developed depressive-like behaviors after experiencing a protocol consisting of 2 hours of daily restraint stress for at least 14 consecutive days.

We found that naive mice had higher *Akap5* messenger RNA levels in the medial prefrontal cortex, hippocampus (HIP), and BLA (Supplemental Figure S3A). Next, we detected the AKAP150 expression in the above brain regions after CRS

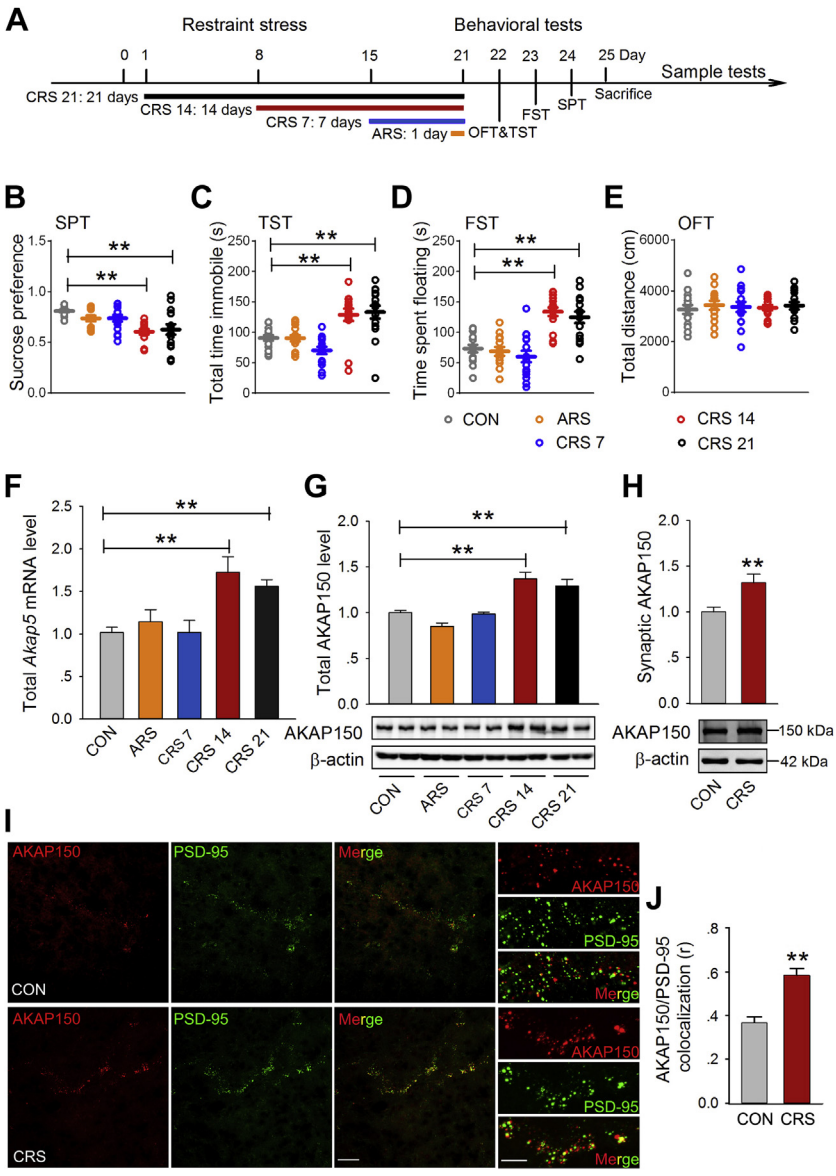

Figure 1. Chronic restraint stress (CRS) increases A-kinase anchoring protein 150 (AKAP150) in the basolateral amygdala (BLA) of mice. **(A)** Restraint stress procedure: mice were restrained for 2 hours/day for 1 day (orange, acute restraint stress [ARS]), 7 consecutive days (blue, CRS 7), 14 consecutive days (red, CRS 14) and 21 consecutive days (black, CRS 21) and subsequently subjected to behavioral tests. Brain tissue samples were obtained on the 25th day. **(B–D)** Mice that underwent the CRS 14 (n = 15) and CRS 21 (n = 15) procedures displayed depressive-like behaviors, including **(B)** reduced sucrose preference in the sucrose preference test (SPT) (one-way analysis of variance [ANOVA] [$F_{4,69}$ = 6.909, p < .01]), **(C)** increased immobile time in the tail suspension test (TST) (one-way ANOVA [$F_{4,69}$ = 12.58, p < .01]), and **(D)** increased floating time in the forced swim test (FST) (one-way ANOVA [$F_{4,69}$ = 18.38, p < .01]). Mice exposed to the ARS (n = 14) or CRS 7 (n = 15) procedure did not show significant behavioral abnormalities compared with control animals (CON) (n = 15). Data are expressed as mean ± SEM. $^{**}p$ < .01. **(E)** Neither ARS nor CRS changed locomotor activity in the open field test (OFT). Data are expressed as mean ± SEM, one-way ANOVA ($F_{4,69}$ = 0.1686, p > .05), n = 14–15. **(F)** Quantitative reverse-transcriptase polymerase chain reaction showed a notable increase in *Akap5* messenger RNA (mRNA) in the BLA of CRS 14 and CRS 21 mice. AKAP150 levels were normalized to control animals. Data are expressed as mean ± SEM, one-way ANOVA ($F_{4,29}$ = 6.893, p < .01), n = 6–8, $^{**}p$ < .01. **(G)** Western blotting showed a significant increase in the expression of AKAP150 in the BLA of CRS 14 and CRS 21 mice. AKAP150 levels were normalized to control animals. Data are expressed as mean ± SEM, one-way ANOVA ($F_{4,35}$ = 18.16, p < .01), n = 7–9, $^{**}p$ < .01. **(H)** Western analysis of synaptic fractions indicated that CRS 14 increased AKAP150 expression in synaptic compartments. Data are expressed as mean ± SEM, unpaired t test (t_{12} = 3.008, p < .01), n = 7, $^{**}p$ < .01. **(I)** Immunolabeling for AKAP150 (red) and postsynaptic density protein 95 (PSD-95) (green) in the BLA slices. Representative confocal images showing increased AKAP150 fluorescence intensity in the postsynaptic density of stressed mice. Scale bar = 40 μm (left) and 10 μm (right). **(J)** Quantification of AKAP150 colocalization with PSD-95 was represented as correlation r value. Data are expressed as mean ± SEM, unpaired t test (t_{18} = 5.666, p < .01), n = 10, $^{**}p$ < .01. Uncropped scans of Western blots are provided in Supplemental Figure S12.

exposure. The results showed that prolonged stressful experience (CRS 14 and CRS 21) selectively increased *Akap5* messenger RNA and protein levels (Figure 1F, G) in the BLA, which were consistent with behavioral outcomes in our study. However, the CRS 14 procedure had no effects on AKAP150 expressions in the medial prefrontal cortex or HIP (Supplemental Figure S3B, C). The amygdala is composed of functionally and morphologically heterogeneous subnuclei with complex interconnectivity, such as the BLA and central nucleus of the amygdala (CeA). We further detected the AKAP150 expression in the CeA and found no change in the CRS model (Supplemental Figure S3D), indicating the specific anatomical upregulation of AKAP150 in the BLA. Based on the above results, the CRS 14 procedure, hereafter referred to as CRS 14, was chosen to further explore the role of AKAP150 in depressive-like behaviors. Synaptic fractionation analysis showed that CRS 14 induced a redistribution of AKAP150 into the synaptic compartment (Figure 1H). Consistently, immunofluorescent staining showed the higher expression and colocalization of AKAP150 with postsynaptic density protein 95 in stressed mice (Figure 1I, J).

In view of different incidences of major depressive disorder between women and men, we also exposed female mice to CRS 14, and notable depressive-like behaviors and anxiety-like behaviors were found (Supplemental Figure S4A–G). Correspondingly, female mice also

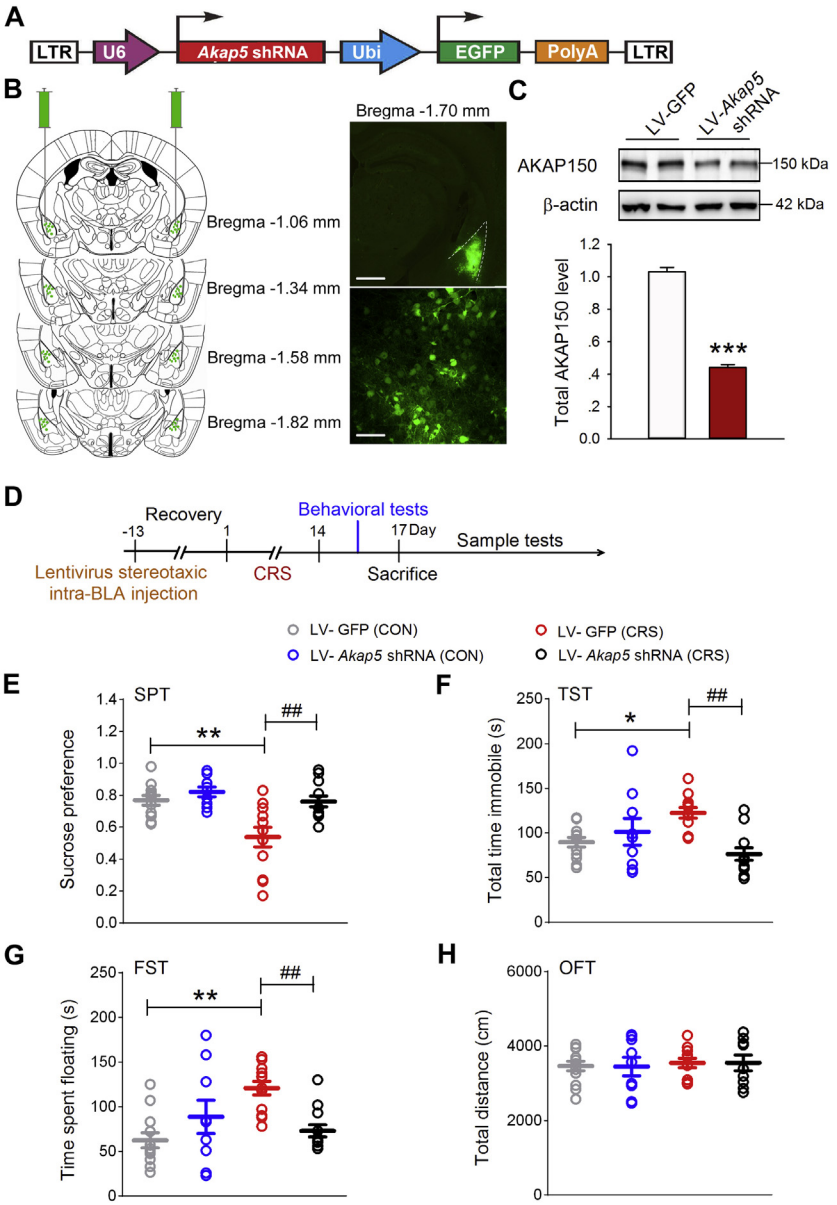

Figure 2. Knockdown of *Akap5* in the basolateral amygdala (BLA) prevents chronic restraint stress (CRS)–induced depressive-like behaviors in mice. **(A)** Lentivirus (LV)-expressing constructs encoding enhanced green fluorescent protein (EGFP) and short hairpin RNAs (shRNAs) targeting *Akap5*. **(B)** The locations of the cannula tips and confocal images of representative fields showed GFP expression in the BLA. Scale bar = 700 μm (upper), 50 μm (bottom). **(C)** Western blot analysis revealed an approximate 58.87% decrease in A-kinase anchoring protein 150 (AKAP150) in the BLA of mice injected with LV-*Akap5* shRNA relative to LV-GFP. Data are expressed as mean ± SEM, unpaired *t* test (t_{18} = 17.47, $p < .001$), n = 10, $^{***}p < .001$. **(D)** Experimental design: mice were recovered for 12 days after injections and then subjected to CRS and subsequently behavioral tests. **(E–G)** Mice that were injected with LV-*Akap5* shRNA showed **(E)** increased sucrose preference in the sucrose preference test (SPT), reduced immobile time in **(F)** the tail suspension test (TST) and **(G)** forced swim test (FST). Data are expressed as mean ± SEM, two-way analysis of variance (SPT: interaction [$F_{1,41}$ = 3.927, $p < .05$], n = 9–12; TST: interaction [$F_{1,41}$ = 12.15, $p < .01$], n = 9–12; FST: interaction [$F_{1,41}$ = 12.81, $p < .01$], n = 9–12). $^{*}p < .05$, $^{**}p < .01$ vs. LV-GFP (control condition [CON]), $^{\#\#}p < .01$ vs. LV-GFP (CRS). **(H)** *Akap5* knockdown in the BLA had no significant effect on mice locomotor activity in the open field test (OFT). Data are expressed as mean ± SEM, two-way analysis of variance, interaction ($F_{1,39}$ = 0.003, $p > .05$), n = 9–12. Uncropped scans of Western blots are provided in Supplemental Figure S12.

showed increased expressions of total and synaptic AKAP150 proteins in the BLA (Supplemental Figure S4H–K), indicating the absence of sex difference in the CRS 14-induced AKAP150 expression. Apart from CRS 14, the other depressive model, chronic unpredictable mild stress (CUMS), was used here. We found that CUMS also induced typical depressive-like behaviors, such as anhedonia and despair behavior (Supplemental Figure S5A–C), followed by elevation of *Akap5* messenger RNA and protein, as well as synaptic AKAP150 in the BLA (Supplemental Figure S5D–H). Taken together, chronic stress increases the expression of AKAP150 and induces its redistribution into the synapses in the BLA of depressive mice.

Knockdown of *Akap5* in the BLA Alleviates Depressive-like Behaviors Induced by CRS 14

To address whether AKAP150 is required for CRS 14-induced depressive-like behaviors, we constructed an effective short hairpin RNA (shRNA) for the specific knockdown of *Akap5* with lentivirus (LV-*Akap5* shRNA) in the BLA (Figure 2A, B). Figure 2C shows the efficiency of LV-*Akap5* shRNA, which decreased the expression of AKAP150 protein by 58.87 ± 3.37%. Twelve days after injection with LVs, mice were exposed to CRS 14 and subsequently to behavioral tests (Figure 2D). As expected, the deficit in the sucrose preference of stressed mice was prevented by *Akap5* shRNA (Figure 2E). As well, knockdown of *Akap5* alleviated CRS 14-induced

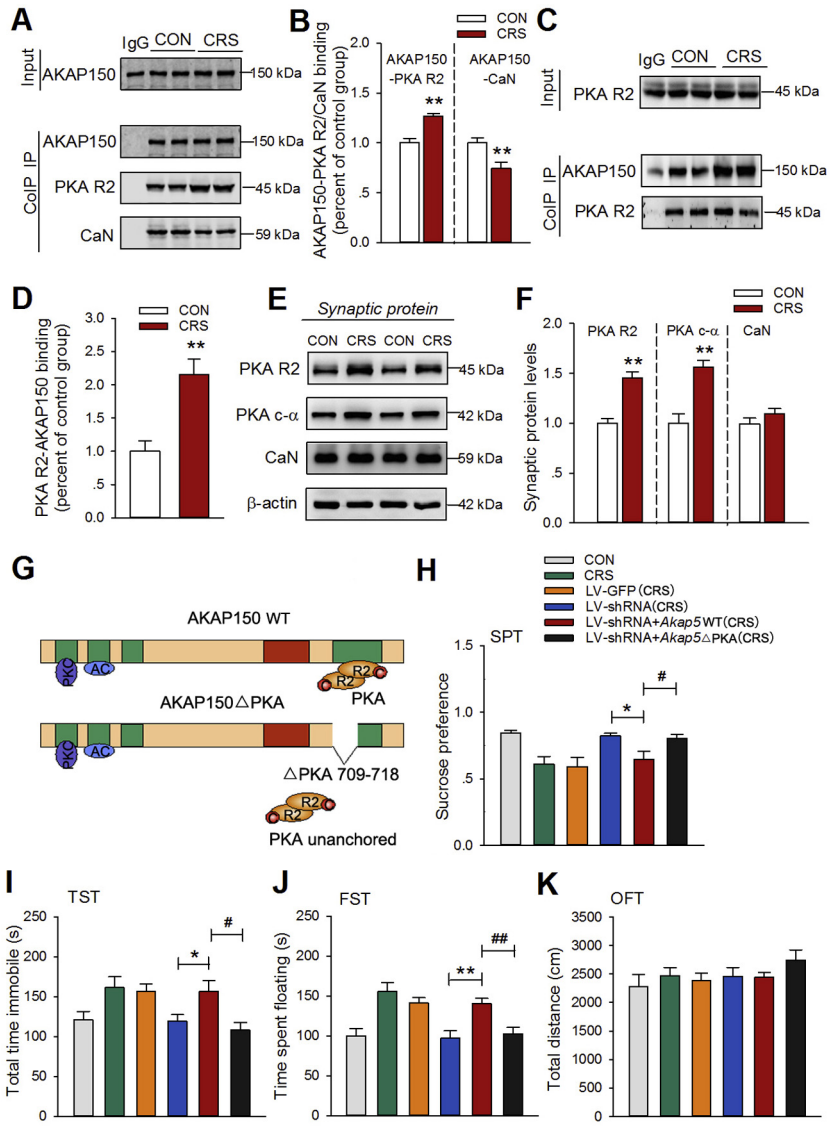

Figure 3. The enhancement of A-kinase anchoring protein 150 (AKAP150) and protein kinase A (PKA) complex signaling contributes to chronic restraint stress (CRS)–induced depressive-like behaviors. **(A, B)** Representative coimmunoprecipitation assay (CoIP) blots and analysis showed an increased association of AKAP150 and PKA regulatory subunit 2 (PKA R2) and a decreased association of AKAP150 and calcineurin (CaN) in the basolateral amygdala (BLA) of CRS mice. Data are expressed as mean ± SEM, unpaired *t* test, AKAP150-PKA R2 ($t_{14} = 4.971$, $p < .01$), $n = 8$, $^{**}p < .01$; AKAP150-CaN ($t_{12} = 3.151$, $p < .01$), $n = 6–8$, $^{*}p < .01$. **(C, D)** Western blotting detected the immunocomplex that was immunoprecipitated by PKA R2 antibody. The data showed an increase in the combination of PKA R2 and AKAP150. Data are expressed as mean ± SEM, unpaired *t* test ($t_{16} = 3.968$, $p < .01$), $n = 9$, $^{**}p < .01$. **(E, F)** Western blotting analysis revealed that CRS promoted PKA subunits to assemble in the synaptic compartment in the BLA. Data are expressed as mean ± SEM, unpaired *t* test, synaptic PKA R2 ($t_{12} = 6.124$, $p < .01$), $n = 7$, $^{**}p < .01$; synaptic PKA catalytic subunit alpha (PKA c-α) ($t_{12} = 4.944$, $p < .01$), $n = 7$, $^{**}p < .01$. The level of CaN in the synaptic compartment was unchanged. Data are expressed as mean ± SEM, unpaired *t* test ($t_{10} = 0.2555$, $p > .05$), $n = 6$. **(G)** Diagram depicting the mouse *Akap5* gene encoding the AKAP150 wild-type (WT) allele (top), the targeting construct containing the ΔPKA mutation (bottom). **(H–J)** The effects of coinjection with LV-*Akap5* short hairpin RNAs (shRNAs) and LV-*Akap5* WT or LV-*Akap5*ΔPKA on CRS-induced depressive-like behaviors in **(H)** the sucrose preference test (SPT) (one-way analysis of variance [ANOVA] [$F_{2,24} = 5.267$, $p < .05$]), **(I)** tail suspension test (TST) (one-way ANOVA [$F_{2,24} = 5.92$, $p < .01$]), and **(J)** forced swim test (FST) (one-way ANOVA [$F_{2,24} = 8.777$, $p < .01$]). **(K)** There were no changes of locomotor activity in the open field test (OFT) (one-way ANOVA [$F_{2,24} = 1.396$, $p > .05$]). Data are expressed as mean ± SEM. $^{*}p < .05$, $^{**}p < .01$ vs. LV-shRNA (CRS), $^{\#}p < .05$, $^{\#\#}p < .01$ vs. LV-shRNA+*Akap5* WT (CRS), $n = 9$. Uncropped scans of Western blots are provided in Supplemental Figure S12. AC, adenylyl cyclase; CON, control condition; IgG, immunoglobulin G; PKC, protein kinase C.

behavioral despair in the TST and FST (Figure 2F, G), with no effect on locomotor activity in the open field test (Figure 2H). These results indicate that the increased AKAP150 in the BLA is required for CRS 14-induced depressive-like behaviors.

CRS 14 Increases the Association of AKAP150 and PKA in the BLA of Mice

AKAPs can direct protein kinases and phosphatases toward their selected substrates to control the content, efficacy, and duration of neuronal phosphorylation events (21). AKAP150 interacts with PKA and CaN to regulate intracellular signaling process (27). Therefore, we first measured the levels of PKA catalytic subunit alpha and PKA regulatory subunit 2 (PKA R2) and CaN in control and stressed mice. Western blotting analysis showed that CRS 14 had no effect on the expression of PKA and CaN in the BLA (Supplemental Figure S6). However, whether the dysfunction of the associations between AKAP150 and PKA and/or CaN plays a role in the development of depressive-like behaviors remains unknown. We next performed coimmunoprecipitation assays to determine which kinase would be involved in CRS 14-induced behavioral changes. Protein complexes were immunoprecipitated by a polyclonal antibody against AKAP150, and immune complexes were validated for PKA R2 and CaN. The result showed an increase in the combination of AKAP150 and PKA R2 and a decrease in the AKAP150 and CaN in the BLA of CRS 14 mice (Figure 3A, B). Meanwhile, protein complexes were immunoprecipitated by a PKA R2 antibody, and immune complexes were detected for AKAP150. The result supported that the association of AKAP150 and PKA R2 in the BLA was increased by CRS 14 (Figure 3C, D). We further examined the

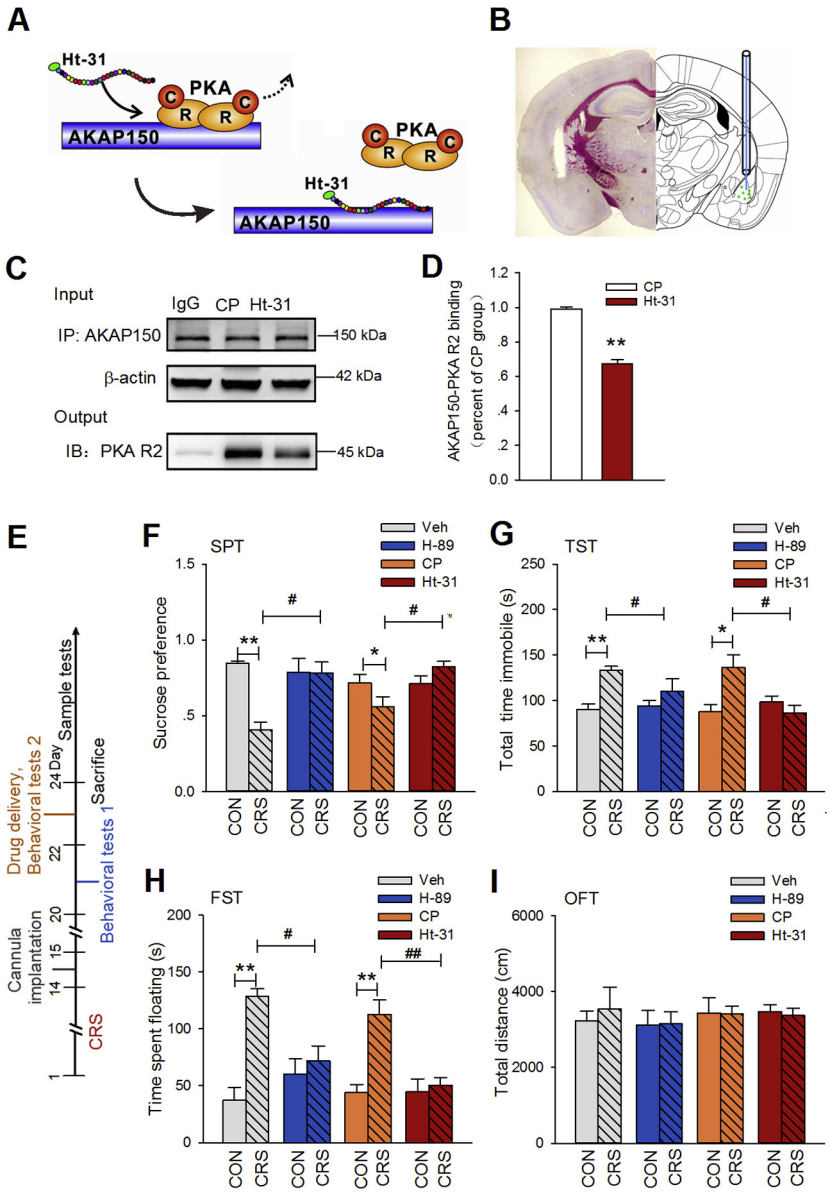

Figure 4. Pharmacological inhibition of A-kinase anchoring protein 150 (AKAP150) and protein kinase A (PKA) signaling rescues depressive-like behaviors of chronic restraint stress (CRS) mice. **(A)** Schematic diagram of Ht-31 inhibitor peptide. Ht-31 peptide was tagged with fluorescein fluorescence. **(B)** Cannula position was histologically verified with Nissl staining. **(C, D)** The verification of interruption between AKAP150 and PKA regulatory subunit 2 (PKA R2) with intrabasolateral amygdala (intra-BLA) injection of 50-μM Ht-31. Data are shown as mean ± SEM, unpaired *t* test ($t_4 = 12.71$, $p < .01$), $n = 3$, $^{**}p < .01$. **(E)** Experimental procedure: mice were injected with drugs through implanted cannulas in the BLA after undergoing CRS. Depressive-like behavioral tests were carried out before (behavioral tests 1) and after drug delivery (behavioral tests 2), and finally, brain tissue samples were obtained. **(F)** Ht-31 and H-89 treatment restored CRS-induced decrease of sucrose preference. Data are shown as mean ± SEM, two-way analysis of variance (ANOVA), CRS × H-89 interaction ($F_{1,32} = 9.203$, $p < .01$), n = 8–10; CRS × Ht-31 interaction ($F_{1,28} = 6.073$, $p < .05$), n = 8. $^{*}p < .05$, $^{**}p < .01$, $^{\#}p < .05$. **(G)** The increment of immobile duration in the tail suspension test (TST) was prevented by Ht-31 and H-89 infusion. Data are shown as mean ± SEM, two-way ANOVA, CRS × H-89 interaction ($F_{1,32} = 9.219$, $p < .01$), n = 8–10; CRS × Ht-31 interaction ($F_{1,28} = 9.831$, $p < .01$), n = 8. $^{*}p < .05$, $^{**}p < .01$, $^{\#}p < .05$. **(H)** Ht-31 peptide and H-89 rescued CRS-induced changes in forced swim test (FST). Data are shown as mean ± SEM, two-way ANOVA, CRS × H-89 interaction ($F_{1,32} = 24.47$, $p < .01$), n = 8–10; CRS × Ht-31 interaction ($F_{1,28} = 9.886$, $p < .01$), $n = 8$. $^{**}p < .01$, $^{\#}p < .05$, $^{\#\#}p < .01$. **(I)** Infusion of H-89 and Ht-31 had no effect on locomotor activity in the open field test (OFT). Data are shown as mean ± SEM, two-way ANOVA, CRS × H-89 interaction ($F_{1,32} = 0.1228$, $p > .05$), n = 8–10; CRS × Ht-31 interaction ($F_{1,28} = 0.02188$, $p > .05$), $n = 8$. Uncropped scans of Western blots are provided in Supplemental Figure S12. CON, control condition; CP, control peptide; IB, immunoblot; IgG, immunoglobulin G; IP, immunoprecipitation; Veh, vehicle.

contents of PKA R2, PKA catalytic subunit alpha, and CaN in the synaptic compartment of BLA region and the larger amounts of both PKA catalytic subunit alpha and PKA R2, but not CaN, were found in the synaptic fraction in CRS 14 mice (Figure 3E, F). Thus, CRS 14 facilitated AKAP150 association with PKA, but not CaN in the BLA, following by depressive-like behaviors.

The Association of AKAP150 With PKA Contributes to CRS 14-Induced Depressive-like Behaviors

The *Akap5* shRNA had a nonspecific effect on the AKAP-anchored PKA versus CaN signaling, both of which would be inhibited by knockdown of *Akap5*. To clarify the role of AKAP150-PKA complex in the depressive-like behaviors, we next constructed an LV vector–expressing AKAP150 (LV-*Akap5* wild-type) or a PKA binding–deficient mutant of AKAP150 (LV-*Akap5*ΔPKA), which specifically deleted the domain that anchors the PKA R2 (28) (Figure 3G). Mice were coinjected with LV-*Akap5* shRNA and LV-*Akap5* wild-type or LV-*Akap5*ΔPKA in the BLA and then exposed to CRS 14 and subsequent behavioral tests. Behavioral results showed that the antidepressant and anxiolytic effects of AKAP150 knockdown in CRS 14 models were abolished by coexpression of AKAP150 wild-type but not AKAP150ΔPKA (Figure 3H–K, Supplemental Figure S7), indicating that the association of AKAP150 with PKA is involved in the CRS 14-induced depressive-like behaviors.

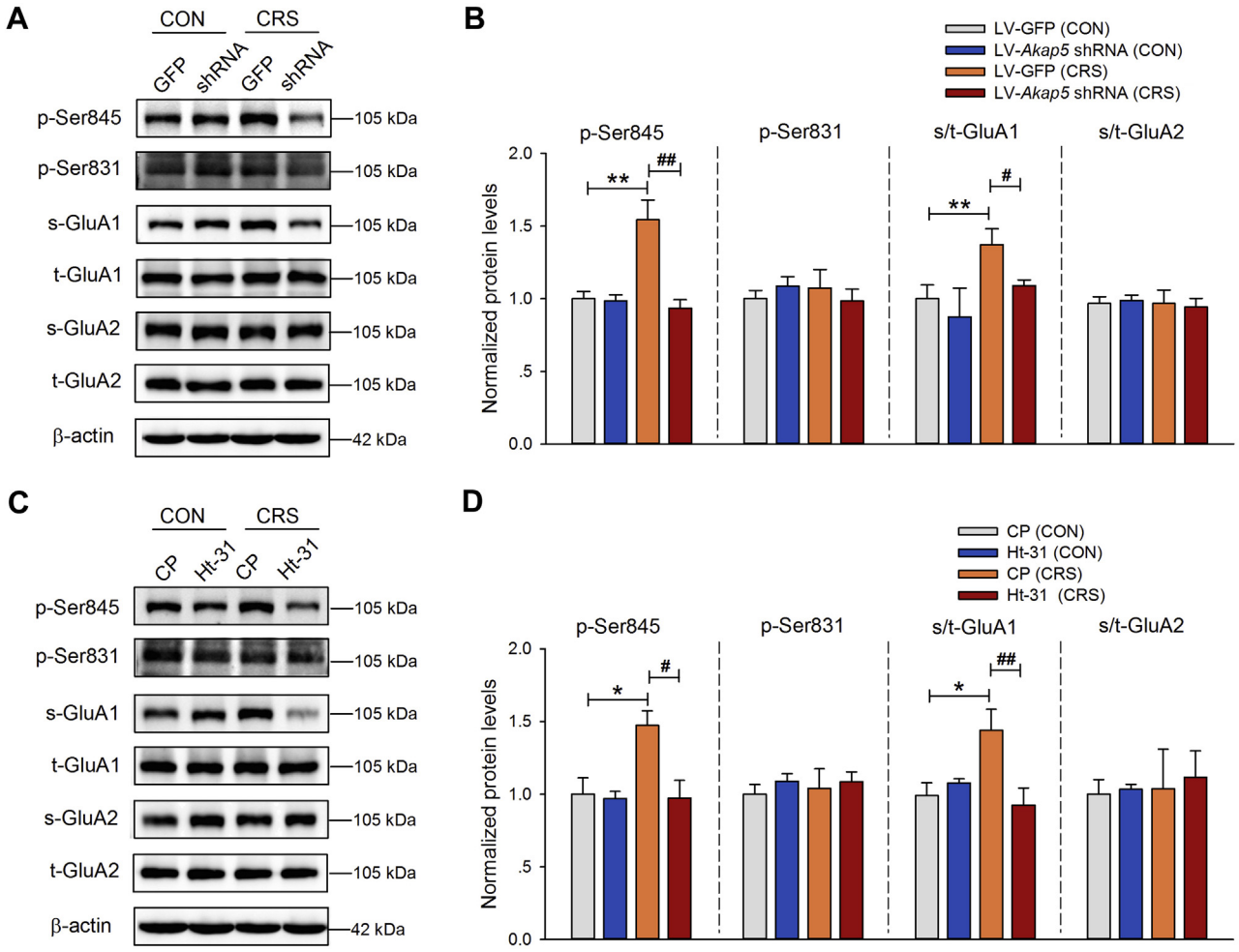

Figure 5. Inhibition of A-kinase anchoring protein 150 and protein kinase A signaling blocks chronic restraint stress (CRS)–induced phosphorylation and membrane insertion of GluA1 in the basolateral amygdala (BLA). **(A, B)** Western blotting results showed the effects of *Akap5* short hairpin RNA (shRNA) on CRS-induced GluA1 phosphorylation and surface expression in the BLA. Data are expressed as mean ± SEM, two-way analysis of variance (ANOVA), phosphorylated serine 845 (p-Ser845): interaction ($F_{1,52} = 13.35$, $p < .01$), $n = 14$; p-Ser831: interaction ($F_{1,36} = 1.088$, $p > .05$), $n = 9$–11. Surface protein/total protein (s/t)-GluA1: interaction ($F_{1,50} = 1.383$, $p > .05$), post hoc test, $n = 13$–14; s/t-GluA2: interaction ($F_{1,32} = 0.1366$, $p > .05$), $n = 9$; $^{**}p < .01$ vs. lentivirus-green fluorescent protein (LV-GFP) (control condition [CON]), $^{\#}p < .05$, $^{\#\#}p < .01$ vs. LV-GFP (CRS). **(C-D)** The effect of Ht-31 on phosphorylation and surface expression of GluA1 under stress. Data are expressed as mean ± SEM, two-way ANOVA, p-Ser845: interaction ($F_{1,32} = 5.538$, $p < .05$), $n = 9$; p-Ser831: interaction ($F_{1,31} = 0.06692$, $p > .05$), $n = 8$–9. s/t-GluA1: interaction ($F_{1,35} = 19.97$, $p < .01$), $n = 9$–10; s/t-GluA2: interaction ($F_{1,24} = 0.01773$, $p > .05$), $n = 7$; $^{*}p < .05$ vs. control peptide (CP) (CON), $^{\#}p < .05$, $^{\#\#}p < .01$ vs. CP (CRS). p-Ser845 and p-Ser831 quantification is normalized to total GluA1 (t-GluA1). Surface protein (s) level is normalized to total protein (t). Uncropped scans of Western blots are provided in Supplemental Figure S12.

Next, we used a well-characterized interference peptide Ht-31 to dissociate PKA from AKAP150 by competing for the binding site (29,30). By conjugating with a fluorescein--fluorescence group, the Ht-31 peptide was traceable, cell permeable, and effective to block the interaction of AKAP150 with PKA in vitro (Supplemental Figure S8) and in vivo (Figure 4A–D). Mice that were subsequently exposed to CRS 14 received bilaterally intra-BLA infusions of Ht-31 or an inactive control peptide. Behavioral tests were performed before and 2 hours after drug infusion (Figure 4E). The results showed that Ht-31 treatment restored the CRS 14-induced deficits in sucrose preference in the SPT and reduced immobility duration in the TST and FST (Figure 4F–H). Given that the PKA signaling in the BLA was activated by CRS 14, we applied H-89 (20 μM), a potent cell-permeable inhibitor of PKA, to inhibit PKA signaling. The experimental procedure was similar to that with Ht-31 treatment. Comparing with vehicle group, H-89 significantly restored the deficit of sucrose preference in the SPT and the behavioral despair in the TST and FST of CRS 14 mice (Figure 4F–H). On the contrary, neither Ht-31 and H-89 had a significant effect on the locomotor activity of mice (Figure 4I). Taken together, these findings suggest that the AKAP150-PKA complex in the BLA is responsible for CRS 14-induced depressive-like behaviors.

Blockage of AKAP150-PKA Signaling Normalizes Phosphorylation and Surface Expression of GluA1 Induced by CRS 14

At the postsynaptic density, PKA is recruited to the neuronal anchoring protein, AKAP79/150 (13,31). AKAP79/150 and its targeted enzymes are directed to AMPARs through association with membrane-associated guanylate kinase protein (13). Furthermore, AKAP79/150-PKA signaling has been shown to regulate the phosphorylation and channel activity of AMPARs (14,32,33). Here, we hypothesized that AKAP150-PKA complex targeting AMPARs would contribute to the CRS 14-induced depressive-like behaviors. To assess this hypothesis, we initially measured the phosphorylated Ser831 (p-Ser831) and Ser845 (p-Ser845) of the GluA1 subunit. It has been demonstrated that Ser-831 is specifically phosphorylated by PKC, while Ser845 is specifically phosphorylated by PKA (34). We found that CRS 14 did not change p-Ser831 level but significantly increased the level of p-Ser845, which was prevented by LV-*Akap5* shRNA (Figure 5A, B). A previous study has reported that these changes in p-Ser845 are paralleled by corresponding changes in the surface expression of AMPARs (16). We thus quantified the surface expression of GluA1 by biotin labeling for cell-surface receptors. Western blotting analysis showed that the increased phosphorylation of Ser845 was accompanied by surface insertion of GluA1, which was prevented by LV-*Akap5* shRNA. On the contrary, the surface level of GluA2 was not affected by CRS 14 or LV-*Akap5* shRNA (Figure 5A, B).

We next asked whether the blockage of the association of AKAP150 and PKA could rescue the changes in phosphorylation and surface expression of GluA1. To address this issue, interference peptide Ht-31 was used for further study. The results showed that Ht-31 prevented the hyperactivity of AMPARs under CRS 14, including increased p-Ser845 and surface insertion of GluA1, but not GluA2 (Figure 5C, D).

AKAP is required for *N*-methyl-D-aspartate receptor–dependent long-term depression via its interaction with PKA and CaN (28,35). To explore the possible contribution of *N*-methyl-D-aspartate receptor, we also examined the effect of *Akap5* shRNA and Ht-31 on the expression of GluN2A/B subunits. The results showed that total and surface GluN2A/B were unchanged by knockdown of AKAP150 or disruption of AKAP150 and PKA (Supplemental Figure S9). Taken together, these results suggest that AKAP150-PKA complex in the BLA contributes to CRS 14-induced depressive-like behaviors via phosphorylation and surface insertion of the GluA1 subunit of AMPARs.

Akap5 shRNA and Ht-31 Rescue CRS 14-Induced Changes in AMPAR-Mediated Synaptic Transmission

To further investigate the effect of CRS 14 on the activity of AMPARs, we measured AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) using whole-cell patch-clamp recordings in BLA slices. The activity of AMPARs was estimated by recording AMPAR-mediated mEPSCs, which were separated by blockade of action potential-evoked glutamate release with tetrodotoxin- and gamma-aminobutyric acid receptor–mediated inhibition with bicuculline. The amplitude of AMPAR-mediated mEPSCs was increased by approximate 30% in the CRS 14 group compared with control animals with LV green fluorescent protein (LV-GFP), and this increase was abolished by injection of LV-*Akap5* shRNA before CRS 14. In contrast to the amplitude of mEPSCs, the frequency was not affected by CRS 14 or *Akap5* shRNA (Figure 6A–C). Next, we asked whether the AKAP150-PKA signaling pathway participated in the CRS 14-elicited enhancement of AMPARs activity. It was shown that Ht-31 prevented the increase in the amplitude of AMPAR-mediated mEPSCs. However, the frequency was unchanged with CRS 14 or Ht-31 (Figure 6D–F). Furthermore, neither the rising time nor the decay time of AMPAR-mediated mEPSCs was altered (Supplemental Figure S10).

Previous studies have shown the involvement of Ca^{2+}-permeable AMPARs and p-Ser845 in stress-related behaviors (36,37). Our immunoblotting results also demonstrated that CRS 14 increased the level of p-Ser845 and surface expression of GluA1. Therefore, we next applied 1-naphthyl acetyl spermine (20 μM), a selective blocker for GluA2-lacking AMPARs, to determine whether selective synaptic incorporation of GluA1 was responsible for an increase in AMPAR-mediated mEPSCs induced by CRS 14. It was found that 1-naphthyl acetyl spermine treatment had no effect on the basal synaptic transmission in control group but significantly reduced the increased amplitude in CRS 14 group, while the frequency, rising time, and decay time were unchanged (Supplemental Figure S11). These results suggest that the increase in AMPA-mEPSC amplitude in the CRS 14 group is mediated by increasing the amount of GluA2-lacking AMPARs. In conclusion, the above results indicate that AKAP150-PKA complex contributes to the increase in AMPAR-mediated mEPSC activity in the BLA of mice exposure to CRS 14 and, at least partially, the incorporation of GluA1 might be responsible for this process.

DISCUSSION

In the present study, we demonstrated that AKAP150-PKA-GluA1 streamline signaling in the BLA mediated the pathophysiological mechanism for depressive-like behaviors induced by chronic stress. Both CRS 14 and CUMS increased AKAP150 level in the BLA and induced a redistribution of AKAP150 into synapses. As the AKAP150 anchoring kinase, PKA was recruited to the synaptic compartment, contributing to the phosphorylation of GluA1 Ser845 site and insertion of GluA1-containing AMPARs into the postsynaptic terminal. Ultimately, the enhanced AMPAR-mediated excitatory synaptic transmission led to depressive-like behaviors (Figure 7). In particular, AKAP150 signaling is well known for the four fundamental tenets: specificity, sensitivity, localization, and temporal control (38). Thus, selective targeting of AKAP150-PKA interaction could be a mean to uncouple pathologically active signaling pathway in depressive disorders with fewer off-target effects.

As a typical AKAP family member in the brain, AKAP79/150 is the best known scaffold protein with the anchoring and targeting properties that are essential for regulating excitatory synaptic transmission and plasticity (12). DNA copy number analysis reveals aberrations in *AKAP5* gene in the postmortem brain of bipolar disorder and schizophrenia patients (22).

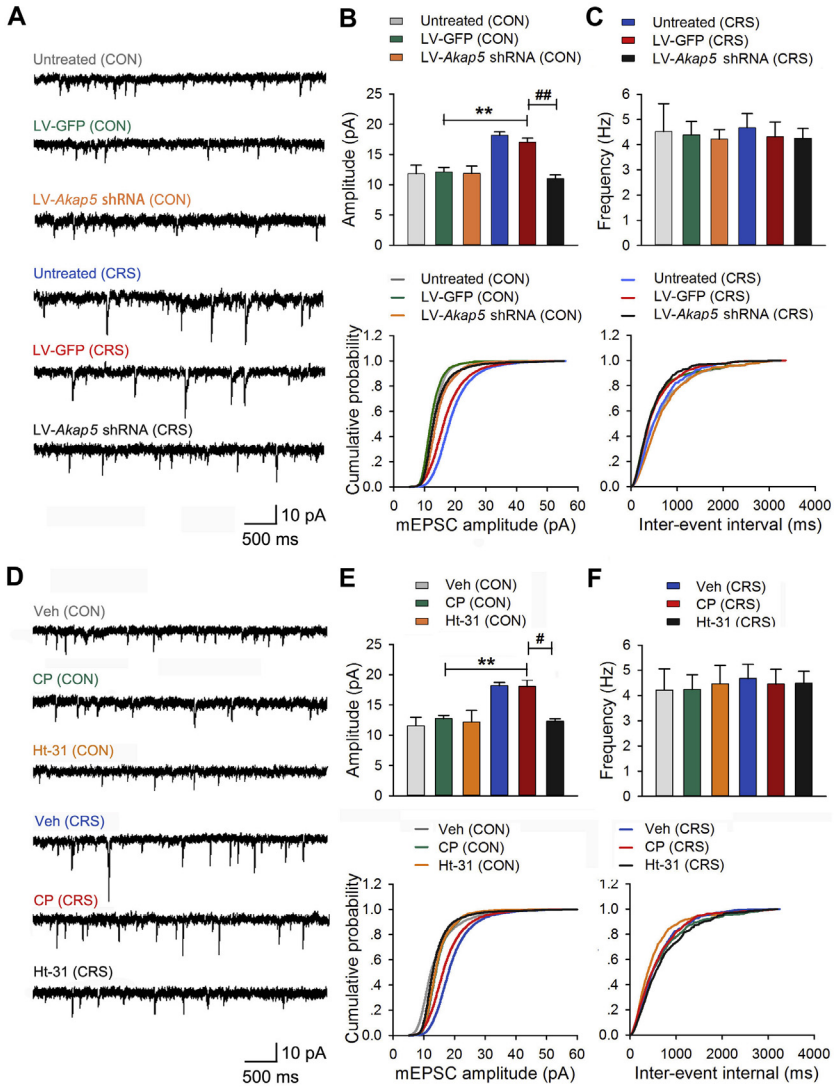

Figure 6. Either *Akap5* short hairpin RNA (shRNA) or Ht-31 peptide restores the increase in amplitude of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptor (AMPAR)–mediated miniature excitatory postsynaptic currents (mEPSCs) induced by chronic restraint stress (CRS). **(A)** Representative traces of AMPAR-mediated mEPSC recordings on the basolateral amygdala (BLA) neuron from untreated (control condition [CON]), lentivirus-green fluorescent protein (LV-GFP) (CON), LV-*Akap5* shRNA (CON), untreated (CRS), LV-GFP (CRS), and LV-*Akap5* shRNA (CRS) mice. **(B)** The statistics of mEPSC amplitude (upper panel) and cumulative probability plots of mEPSC amplitude for representative cells from each group (lower panel). Knockdown of *Akap5* reduced mEPSC amplitude by ~30% relative to scrambled vector injection in stressed mice (LV-GFP [CRS]). Data are expressed as mean ± SEM, two-way analysis of variance (ANOVA), interaction ($F_{2,48}$ = 8.745, $p < .01$), post hoc test. $^{**}p < .01$ vs. LV-GFP (CON), $^{\#\#}p < .01$ vs. LV-GFP (CRS), n = 9 brain slices from 5–6 mice per group. **(C)** The statistics of mEPSC frequency (upper panel) and cumulative probability plots of mEPSC frequency for representative cells from each group (lower panel). The frequency of AMPAR-mediated mEPSCs was not affected by CRS and *Akap5* shRNA expression. Data are mean ± SEM, two-way ANOVA, interaction ($F_{2,48}$ = 0.01704, $p > .05$), n = 9 brain slices from 5–6 mice per group. **(D)** Representative recordings of AMPAR-mediated mEPSCs from the vehicle (Veh) (CON), control peptide (CP) (CON), Ht-31 (CON), Veh (CRS), CP (CRS), and Ht-31 (CRS) groups. **(E)** The statistics of mEPSC amplitude (upper panel) and cumulative probability plots of mEPSCs amplitude for representative cells from each group (lower panel). Ht-31 peptide prevented CRS-induced increase in mEPSC amplitude. Data are represented as mean ± SEM, two-way ANOVA, interaction ($F_{2,48}$ = 4.829, $p < .05$), post hoc test. $^{**}p < .01$ vs. CP (CON), $^{\#}p < .01$ vs. CP (CRS), n = 9 brain slices from 5–6 mice per group. **(F)** The statistics of frequency (upper panel) and cumulative probability plots of mEPSC frequency for representative cells from each group (lower panel). Ht-31 infusion did not affect mEPSC frequency. Data are as mean ± SEM, two-way ANOVA, interaction ($F_{2,48}$ = 0.05937, $p > .05$), n = 9 brain slices from 5–6 mice per group.

Recently, increasing evidence demonstrates AKAP79/150 signaling may play a role in pathophysiology of seizures, addiction, neurodegenerative diseases, and bipolar disorders (23,39,40). Growing evidence from genetic, molecular, and pharmacological manipulations has indicated that AKAP79/150 may also be involved in the neuropsychiatric disorder (22,23), but little is known about its role of in the depressive disorder. In our study, we found that depressive-like behaviors were accompanied by an increase in AKAP150, specifically in the BLA of stressed mice. As certain responses to chronic stress are adaptive and beneficial to the survival of the animal, which may lead to habituation to repeated stress exposure, the CUMS model was used here to exclude the homotypic stressors. The increased AKAP150 expression in the CUMS model indicated that, at least partially, AKAP150 signaling in stress-related depressive-like behavior was not resulted from habituation to the stressor. In addition, knockdown of *Akap5* in the BLA prevented CRS 14-induced depressive-like behaviors. Our results provide evidence for AKAP150 as a crucial novel key regulator of stress susceptibility.

The most common pattern of AKAP150 is to anchor with PKA, PKC, and CaN, controlling the activity of AMPARs via phosphorylation and dephosphorylation events (12). In the current study, we found that the interaction of AKAP150 with PKA but not with CaN was significantly increased in the CRS 14 model. Furthermore, inhibition of PKA activity with H-89 and blockage of binding of AKAP150 and PKA with Ht-31 reversed depressive-like behaviors in CRS 14 mice, suggesting that AKAP150 anchoring PKA was involved in CRS 14-induced depressive-like behaviors. Numerous studies support the model that AKAP79/150 coordinately regulates the activity of the GluA1 through the opposing actions of PKA and CaN

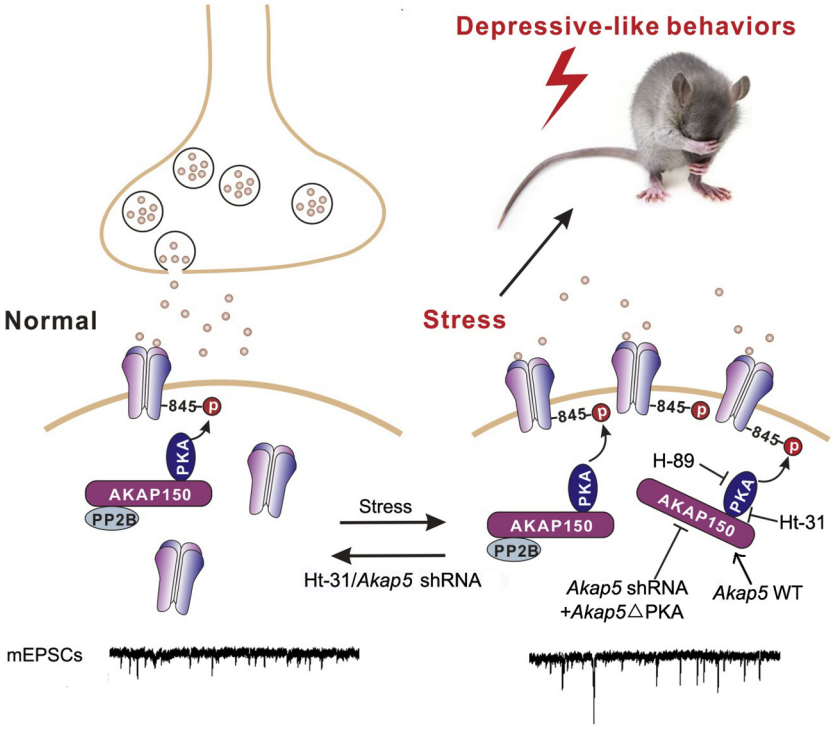

Figure 7. Diagram depicting the involvement of A-kinase anchoring protein 150 (AKAP150) and protein kinase A (PKA) signaling complex in chronic restraint stress–induced depressive-like behaviors. Chronic restraint stress increases phosphorylation of GluA1 at serine 845 by AKAP150 anchoring PKA, leading to alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptors insertion into postsynaptic terminal, which strengthens excitatory synaptic transmission in the basolateral amygdala and induces depressive-like behaviors in mice. In turn, knockdown of *Akap150* or inhibition of the association between AKAP150 and PKA rescues the changes in synaptic transmission and behaviors induced by chronic restraint stress. p, phosphorylation; mEPSC, miniature excitatory postsynaptic current; PP2B, protein phosphatase 2B; shRNA, short hairpin RNA; WT, wild-type.

(12,14,41). Our result is consistent logically with the previous finding that downregulation of CaN in the amygdala is sufficient to induce depressive-like behaviors in mice (42). Likewise, AKAP79/150 also anchors PKC to phosphorylate GluA1 (43). Different from PKA phosphorylation site at Ser845, AKAP79/150-anchored PKC is capable of regulating GluA1-mediated currents through phosphorylation of Ser831 (12). Our results showed that CRS 14 significantly increased the phosphorylation level of GluA1 at the PKA-dependent site Ser845 rather than PKC-dependent site Ser831 in the BLA, indicating that AKAP150-anchored PKC was not activated following CRS 14.

It has been reported that AKAP150-anchored PKA phosphorylation event plays a key role in controlling the synaptic expression of AMPARs, and the changes in Ser845 phosphorylation are paralleled by corresponding changes in the surface expression of GluA1-containing AMPARs (21). Here, we found that both AKAP150 and PKA were increased in synaptic compartments under CRS 14, and the phosphorylation and surface insertion of GluA1 were promoted correspondingly. Meanwhile, changes in Ser845 phosphorylation and membrane fraction of GluA1 in CRS 14 mice were reversed by intra-BLA injection of LV-*Akap5* shRNA or Ht-31 peptide. Therefore, AKAP150-PKA signaling complex might be the vital regulator of GluA1-containing AMPARs in the BLA under chronic stress.

Despite the enrichment of PKA in the synaptic compartment, the mechanism responsible for the activation of PKA signals following CRS 14 remains unknown. Norepinephrine is an essential stress hormone to modulate stress response. The BLA receives dense noradrenergic projections from the locus coeruleus, and norepinephrine is increased in the BLA in response to aversive stress (44). Activation of β-adrenoceptors by norepinephrine results in the elevation of the intracellular cAMP concentration and subsequent PKA activation, contributing to the facilitation of excitatory synaptic transmission in the lateral amygdala (45). In addition, the BLA expresses dopamine receptors and receives dopamine innervation from the ventral tegmental area (46,47). Dopamine levels in the amygdala are highly responsive to acute stress (48). The D_1-receptor activation is associated with an increase in cAMP/PKA signaling (49), and D_1/PKA signaling pathway plays a critical role in improving the HIP-dependent spatial memory via AMPAR trafficking and synaptic plasticity (50,51). In our study, CRS 14-induced excessive excitatory synaptic transmission required the redistribution of PKA anchored by AKAP150 and PKA-mediated phosphorylation of the AMPARs. Thus, activation of β-adrenoceptors and dopamine D_1 receptor may account for the activation of PKA under the stress condition, which needs to be further identified.

Chronic stress increases the excitability of BLA neurons and contributes to the expression of pathological stress behavior, such as depression and anxiety (52). Alteration of neuronal activity has been considered as a form of plasticity when facing stress. Synaptic recruitment of Ca^{2+}-permeable AMPARs plays a crucial role in activity-dependent synaptic plasticity. Here, we found that the amplitude of AMPAR-mediated mEPSCs was increased in the CRS 14 group, the incorporation of GluA1 might be partially responsible for this process, and disruption of AKAP150-PKA complex normalized the changes in AMPAR-mediated mEPSCs. Thus, chronic stress facilitated AMPAR, mainly GluA1-mediated

glutamatergic neurotransmission via AKAP150-anchored PKA complex signaling. The enhanced glutamatergic neurotransmission may account for stress-induced alterations in neuronal response. It is worth noting that circuit-specific actions of BLA neurons have been identified in recent studies (53). The BLA receives sensory information about the external environment and is reciprocally connected with the prefrontal cortex, HIP, nucleus accumbens, and CeA (54). BLA-nucleus accumbens and BLA-CeA projections have been found to encode positive and negative valence, respectively (55,56). Furthermore, the BLA-HIP circuit is critical in governing anxiety-related behaviors (57,58). Therefore, the circuit/project of BLA neurons related to AKAP under the chronic stress condition needs to be further investigation.

In conclusion, we found that the AKAP150-PKA complex in the BLA was critical for modulating depressive-like behaviors. It was possible that AKAP150 anchoring PKA induced a long-lasting increase in phosphorylation and surface expression of GluA1 that strengthened the synaptic connection, which could, at least in part, explain the increased BLA activity under the exposure to chronic stress (57,59). These results reveal insight into the pathophysiology of depression and provide a novel target for refining therapeutic approaches to depressive disorders.

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H-YZ and J-GH primarily performed most of the experiments. Z-LH conceived the project and designed the experiments. Z-LH and S-GX contributed to the electrophysiology recordings. J-FX performed the coimmunoprecipitation assay experiments. Q-QC carried out immunohistochemistry experiments on slices. S-QG, BZ, P-FW, and L-HL participated in the behavioral tests and qPCR examination. J-GC and FW supervised the project, revised the manuscript and supported funding acquisition.

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ARTICLE INFORMATION

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