Molecular Psychiatry (2018) 23, 556–568
www.nature.com/mp

OPEN

ORIGINAL ARTICLE

Gene deficiency and pharmacological inhibition of caspase-1 confers resilience to chronic social defeat stress via regulating the stability of surface AMPARs

M-X Li[1], H-L Zheng[1], Y Luo[1], J-G He[1], W Wang[1], J Han[1], L Zhang[1], X Wang[1], L Ni[1], H-Y Zhou[1], Z-L Hu[1,2,3], P-F Wu[1,2,3], Y Jin[1,2], L-H Long[1,2,3], H Zhang[1], G Hu[4], J-G Chen[1,2,3,5,6] and F Wang[1,2,3,5,6]

Both inflammatory processes and glutamatergic systems have been implicated in the pathophysiology of mood-related disorders. However, the role of caspase-1, a classic inflammatory caspase, in behavioral responses to chronic stress remains largely unknown. To address this issue, we examined the effects and underlying mechanisms of caspase-1 on preclinical murine models of depression. We found that loss of caspase-1 expression in *Caspase-1*$^{-/-}$ knockout mice alleviated chronic stress-induced depression-like behaviors, whereas overexpression of caspase-1 in the hippocampus of wild-type (WT) mice was sufficient to induce depression- and anxiety-like behaviors. Furthermore, chronic stress reduced glutamatergic neurotransmission and decreased surface expression of glutamate receptors in hippocampal pyramidal neurons of WT mice, but not *Caspase-1*$^{-/-}$ mice. Importantly, pharmacological inhibition of caspase-1-interleukin-1β (IL-1β) signaling pathway prevented the depression-like behaviors and the decrease in surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) in stressed WT mice. Finally, the effects of chronic stress on both depression- and anxiety-like behaviors can be mimicked by exogenous intracerebroventricular (i.c.v.) administration of IL-1β in both WT and *Caspase-1*$^{-/-}$ mice. Taken together, our findings demonstrate that an increase in the caspase-1/IL-1β axis facilitates AMPAR internalization in the hippocampus, which dysregulates glutamatergic synaptic transmission, eventually resulting in depression-like behaviors. These results may represent an endophenotype for chronic stress-induced depression.

Molecular Psychiatry (2018) **23,** 556–568; doi:10.1038/mp.2017.76; published online 18 April 2017

INTRODUCTION

Major depressive disorder (MDD) is highly prevalent and characterized by profound disturbances in emotions, motivation, cognition and physiological function. MDD is a significant cause of mortality and morbidity worldwide. Although MDD exhibits multiple molecular phenotypes,[1,2] there are various competing and sometimes overlapping insights into the physiological mechanisms that underlie the complexities of MDD. The inflammatory hypothesis is one of the most important mechanisms for the pathophysiology of depression. Both clinical trials and meta-analysis have consistently demonstrated increased serum levels of proinflammatory cytokines among depressed clients.[3–5] It is well known that the interleukin-1β (IL-1β)-converting enzyme caspase-1 is an inflammatory caspase, and activation of caspase-1 involves the response of immune cells to both pathogen-derived and endogenous mediators by the formation of inflammasome complexes. Caspase-1 has been demonstrated to have a role in inflammatory forms of cell death and protein cleavage and also in protein secretion.[6] Interestingly, the level of caspase-1 mRNA is increased in periphery blood mononuclear cells in MDD patients when compared with health individuals, and can be reduced by treatment with antidepressants.[7] Inflammatory cytokines may induce both peripheral and central action. A recent study has demonstrated that genetic deficiency or pharmacological inhibition of caspase-1 prevents chronic restraint stress (CRS) -induced depressive-like behaviors, involving a modulation of the relationship between stress and gut microbiota composition.[8] In addition to its role in peripheral inflammation, growing evidences also indicate that caspase-1-mediated neuroinflammation in the brain mediates depression-like behavior induced by lipopolysaccharide or chronic stress.[9–11] However, how the increased caspase-1 in the brain influences the depressive-like behavior remains largely unknown.

Several circumstantial lines of evidence have shown that glutamatergic neurotransmission is directly or indirectly involved in the pathophysiology and treatment of mood disorders.[12,13] Glutamatergic abnormalities have been observed in plasma, cerebrospinal fluid (CSF) and brain tissue of individuals with depression, and treatment with antidepressants can decrease plasma levels of glutamate in depressed individuals.[14,15] Postmortem studies have shown a reduced expression of excitatory amino acid transporter 1 (EAAT1) and EAAT2, which have an essential role in maintaining proper neuronal function by modulating glutamate recycling at the synapse in the prefrontal

[1]Department of Pharmacology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [2]The Key Laboratory for Drug Target Researches and Pharmacodynamic Evaluation of Hubei Province, Wuhan, China; [3]Laboratory of Neuropsychiatric Diseases, The Institute of Brain Research, Huazhong University of Science and Technology, Wuhan, China; [4]Department of Pharmacology, Nanjing University of Chinese Medicine, Nanjing, China; [5]The Collaborative-Innovation Center for Brain Science (HUST), Wuhan, China and [6]The Key Laboratory of Neurological Diseases (HUST), Ministry of Education of China, Wuhan, China. Correspondence: Professor J-G Chen or Professor F Wang, Department of Pharmacology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan 430030, China.
E-mail: chenj@mails.tjmu.edu.cn or wangfangtj0322@163.com
Received 28 April 2016; revised 23 February 2017; accepted 24 February 2017; published online 18 April 2017

cortex.[16] Meanwhile, dysfunction of glutamatergic neurotransmission in rodents is associated with anhedonia, impaired social interactions (SIs), weight loss, cognitive impairment, behavioral despair and dysregulated feeding behavior.[12,17–20] For example, chronic unpredictable mild stress (CUMS) selectively attenuates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) -mediated synaptic transmission in the hippocampus of rats.[21] Chronic administration of AMPA produces antidepressant effects in both the forced swim and sucrose preference test by increasing hippocampal brain-derived neurotrophic factor (BDNF).[22] In addition, GluA1 knockout (*Gria1*$^{-/-}$) mice exhibit a depression-like phenotype and increased levels of glutamate in the hippocampus,[23] but not in forebrain excitatory neurons.[24] Collectively, these observations indicate that glutamatergic system is a primary mediator of depression pathology and, potentially, also a final common pathway for the therapeutic action of antidepressant agents.

It has been found that inhibition of caspase-1 significantly enhances AMPAR-mediated long-term potentiation (LTP) without affecting the *N*-methyl-D-aspartate receptor (NMDAR) -mediated component.[25] Conversely, elevated IL-1β levels decrease the surface expression of AMPARs and inhibits LTP in the hippocampus.[26,27] and may underlie the pathophysiology of various psychiatric disorders, such as depression, anxiety and addiction.[28–30] Interestingly, ketamine, a new type of antidepressant that is dependent on the activation of AMPARs,[31] also reduces the levels of proinflammatory cytokines in adult rats following maternal deprivation.[32] Furthermore, recent studies have shown that neuroinflammation regulates CUMS-induced impairment of cognition and LTP by attenuating GluA1 phosphorylation.[33] However, it remains unclear whether there is a causal relationship between caspase-1-mediated regulation of AMPAR-dependent neurotransmission and depression-like behavior.

In this study, we hypothesized that chronic stress may increase the level of caspase-1 in the hippocampus, which would contribute to the internalization of AMPAR, eventually leading to the impairment of synaptic transmission and depression-like behavior (Figure 1a). Thus, we performed a variety of behavioral, immunofluorescence, molecular and electrophysiological experiments to investigate whether the caspase-1 contributes to the induction of depression-like behaviors by regulating the surface stability of AMPARs in animal models.

MATERIALS AND METHODS

Animals

Adult male C57BL/6J mice (7–9 week old, 22–24 g) were purchased from Hunan SJA Laboratory Animal (Changsha, Hunan, China). Both caspase-1 knockout (*Caspase-1*$^{-/-}$) and their wild-type (WT) mice on a C57BL/6J genetic background were obtained from Prof. Gang Hu (Nanjing Medical University, Nanjing, Jiangsu, China), using an identical strategy as described previously.[34] Mice were bred and maintained in the Animal Resource Centre of the Faculty of Medicine, Huazhong University of Science and Technology. *Caspase-1*$^{-/-}$ mice were generated by crossing germline-heterozygous-null mutant *Caspase-1*$^{+/-}$ mice. The offspring were genotyped by PCR with reverse transcriptase using mouse tail-tip DNA and common primers (5′-GAA GAG ATG TTA CAG AAG CC-3′), WT primers (5′-CAT GCC TGA ATA ATG ATC ACC-3′) for 600 bp and mutant allele-specific primers (5′-GCG CCT CCC CTA CCC GG-3′) for 600 bp. The PCR products were visualized with ethidium bromide staining. The C57BL/6J mice were housed in groups of four or five per cage in plastic cages, and maintained under standard laboratory conditions (12:12 light cycle at a constant temperature (22 ± 2 °C) with free access to food and water) unless otherwise indicated. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Welfare Committee of Huazhong University of Science and Technology.

Stress procedures

Chronic social defeat stress (CSDS) and SI were performed as previously reported.[35] For CSDS protocol, a singly C57BL/6J intruder mouse was exposed to a different CD1 aggressor mouse for 5–10 min each day for a total of 10 days. Following 5–10 min of contact, the intruder C57BL/6J mouse was housed across a perforated plexiglass divider providing further stressful sensory cues from the aggressor CD1 mouse for the remainder of the 24 h period (Supplementary Figure 1). Control C57BL/6J mice were pair housed in defeat boxes with one mouse per side of the perforated divider. All control mice that were placed with the controls were changed daily. On day 11, defeated animals were subjected to the SI test and sorted into either susceptible or resilient phenotypes based on interaction scores.

SI test was composed of two 150 s phases, a highly reliable indicator of depressive-like behaviors. All data during these phases were automatically recorded by AniLab software (AniLab Software & Instruments, Ningbo, China). During the first phase, the C57BL/6J mouse was allowed to explore freely in a square-shaped open-field arena (45 × 45 × 45 cm) possessing a wire-mesh cage (10 × 6 cm) opposed to one side, with their movement tracked in the absence of the aggressor. In the second phase, the mouse was reintroduced into this arena with an unfamiliar CD1 mouse within the cage. After each phase, the apparatus was cleaned with a solution of 70% ethanol in water to remove olfactory cues and all of the behavioral tests were conducted under red-light conditions in a room isolated from external sound sources. According to the time spent in the interaction zone (14 × 26 cm) and corner zone (10 × 10 cm) during the first (no target) and second (target) phase, the interaction ratio was calculated as 100 × (time spent in the interaction zone with an aggressor)/(time spent in the interaction zone without an aggressor) and an interaction ratio of 100 was set as a cutoff: the mice with scores < 100 were considered susceptible and those with scores $\geqslant 100$ were considered resilient.

CUMS was performed as previously described.[36] Mice were singly caged and habituated to 1% sucrose solution for 2 days as described later in this section. Mice were then subjected to a CUMS protocol (Supplementary Table 1). Briefly, this protocol consisted of the sequential application of a variety of mild stressors, including cold (4 °C for 1 h), wet bedding (12 h), swim stress (18 °C for 10 min), reversal of the light–dark cycle, no bedding (12 h), restraint (1 h), cage tilting (45°, 12 h), foot shock (0.5 mA, 10–20 times, 5–30 s intervals), strobe light (12 h) and food and water deprivation (24 h). All stressors were randomly interspersed throughout the stress period and this schedule lasted for 5 weeks.

CRS was conducted based on previously described procedures with minor modifications.[17] Mice were subjected to restraint stress by placement for 3–4 h per day in 50 ml conical tubes with containing 12 0.5 cm holes for airflow at 1000 h. This protocol was processed for 10 consecutive days. During CRS, all mice experienced no physical suppression or pain experience, and were placed in separate sound- and light-attenuating boxes and then immediately returned to their home cages. The four experimental groups were referred to as the WT, WT-CRS, *Caspase-1*$^{-/-}$ and *Caspase-1*$^{-/-}$-CRS.

Mouse behavioral tests

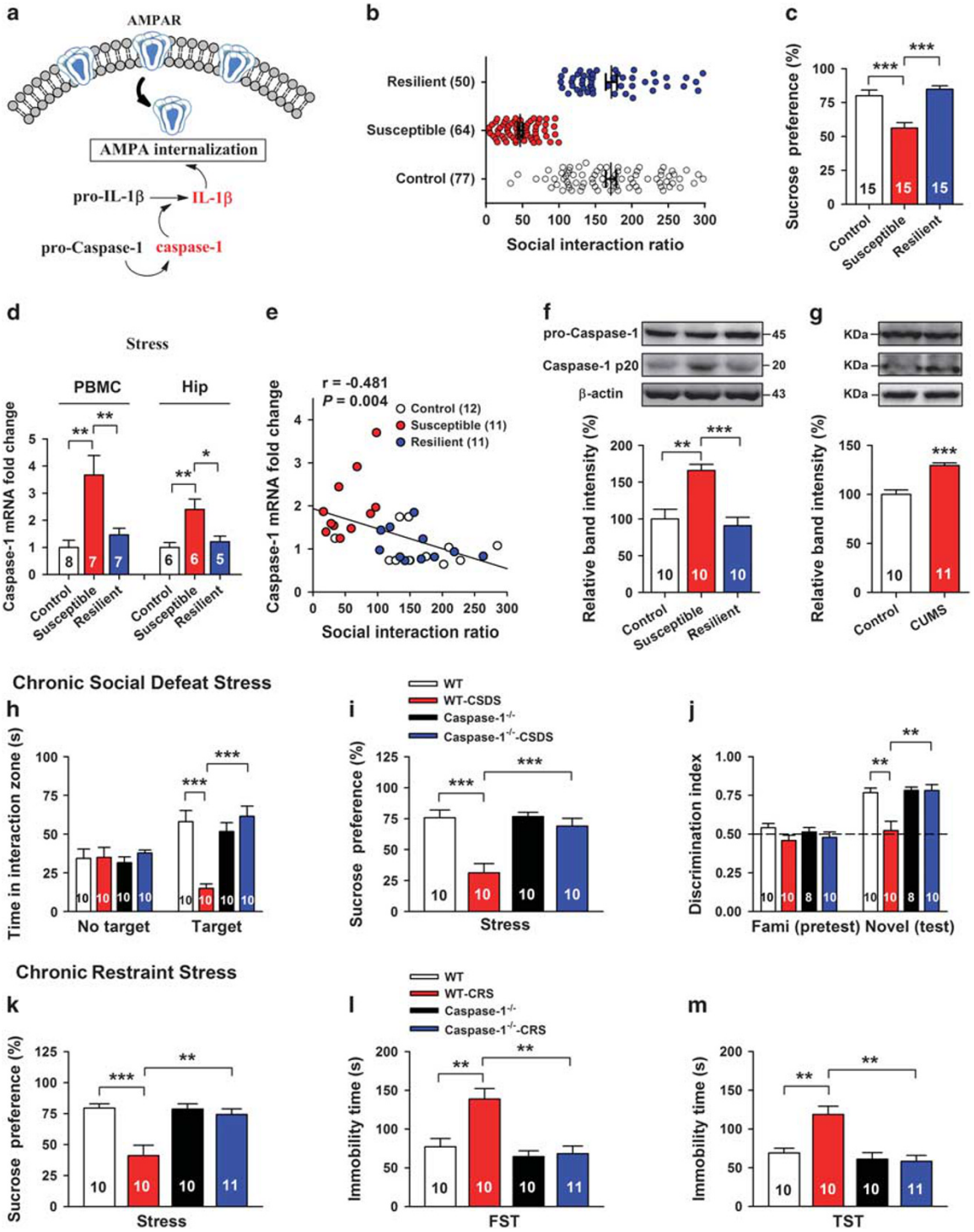
All mouse behavioral tests were recorded using a videotracking system (AniLab software; AniLab Software & Instruments) unless otherwise indicated. All animals were transported to a dimly illuminated behavioral room and were left undisturbed for at least 1 h before testing. Detailed materials and methods of the novel object recognition, sucrose preference test, tail suspension test (TST), forced swim test (FST), measurement of pain threshold, open-field test (OF) and elevated plus maze (EPM) were provided in the Supplementary Information. Depression-like behaviors were assessed using SI, sucrose preference test, TST and FST, and OF and EPM for anxiety-like behaviors. We used multiple behavior testing on the same animals. All animals from different groups were given the same tests in the same order.

Electrophysiological recordings in hippocampal slices

Whole-cell patch-clamp recordings. C57BL/6 J (10–12 weeks) mice were anaesthetized with isoflurane, and then perfused with 30 ml ice-old oxygenated (95% O_2 and 5% CO_2) dissection buffer that contained (in mM): 210 sucrose, 3.1 sodium pyruvate, 11.6 sodium L-ascorbate, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 5.0 $MgCl_2$ and 20.0 glucose, pH 7.4 (300 mOsm). Brains were removed rapidly into oxygenated chilled dissection buffer. Coronal hippocampal slices (300 μm) were prepared using a vibratome (VT 1000 S, Leica, Wetzlar, Germany), and the slices were incubated for

at least 1.5 h in an interface chamber containing artificial cerebrospinal fluid that consisted of (in mM): 119.0 NaCl, 3.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$ and 11.0 glucose, pH 7.4 (300 mOsm). Whole-cell voltage-clamp recordings of CA1 pyramidal cells were made in a submersion chamber with patch electrodes (3-6 MΩ resistance) filled with a solution containing (in mM): 122.5 Cs-gluconate, 17.5 CsCl, 0.2 EGTA, 10.0 HEPES, 1.0 $MgCl_2$, 4.0 Mg-ATP, 0.3 Na-GTP, and 5.0 QX314, pH 7.2 (280-300 mOsm). AMPAR-mediated miniature excitatory postsynaptic current (mEPSC) was isolated by including bicuculline (20 μM) and tetrodotoxin (1 μM) in the bath solution. All recordings were performed at a holding potential of -70 mV with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) under an upright Olympus microscope (BX51WIF, Olympus, Tokyo, Japan) at room temperature. Data was filtered at 2 kHz and sampled at 10 kHz using Digidata 1322A digitizer (Molecular Devices), and then was acquired with pClamp 10 software. Data was analyzed by Mini Analysis Program (Synaptosoft, Decatur, GA, USA) with an amplitude threshold of 5 pA for mEPSC analysis.

Field potential recording. Brain slices were obtained according to our previous studies with some modifications.[37] Briefly, the brain was removed immediately after the anaesthetized animal was decapitated and coronal hippocampal slices (350 μm) were cut using Leica VT 1000 S vibratome in



ice-cold artificial cerebrospinal fluid. After 1.5 h of recovery at room temperature, an individual slice was transferred to a submerged recording chamber and continuously superfused with oxygenated artificial cerebrospinal fluid at 30 ± 1 °C at a rate of 2–4 ml min^{-1}. Field excitatory postsynaptic potentials (fEPSPs) were evoked by a constant stimulation in the Schaffer collaterals with a bipolar stimulating electrode and recorded in the stratum radiatum layer of CA1 region with a glass micropipette (3–5 MΩ resistance) filled with 3 M NaCl. Test frequency to evoke fEPSPs was 0.03 Hz. The amplitude of the fEPSPs was usually set at 30–35% of maximal responses. The input–output relationship for synaptic transmission was recorded by stimulating Schaffer collateral-CA1 afferents with increasing levels of intensity. When paired-pulse facilitation was determined, paired stimuli (25, 50, 75, 100 and 200 ms intervals) were delivered and the ratio of the slope of the second fEPSP (fEPSP2) over the first one (fEPSP1) was determined. After recording of stable baseline for at least 20 min, LTP was induced by three trains of high-frequency stimulation (HFS: 100 pulses at 100 Hz separated by 30 s). LTD was induced by single-pulse low-frequency stimulation (LFS: 900 pulses at 1 Hz for 900 s) and the stimulation intensity was adjusted to give fEPSP slopes of ~50% of maximum. The fEPSP was monitored for 60 min and the fEPSP slopes were normalized to the average of the slopes of the fEPSPs acquired during the baseline. All data were calculated as the average of the last 15 min out of 60 min recordings.

Stereotaxic surgery. C57BL/6J mice were anesthetized with sodium pentobarbital (45 mg kg^{-1}, i.p.) and then placed in a stereotaxic apparatus. 22-gauge stainless steel guide cannulas were unilaterally implanted in the intracerebroventricular region (AP = − 0.2 mm, ML = ± 0.9 mm, DV = − 2.4 mm). C57BL/6J mice were individually housed, and allowed to recover for at least 5 days after surgery. AC-Tyr-Val-Ala-Asp-Chloromethylketone (AC-YVAD-CMK) (5 or 10 μg kg^{-1}) (dissolved in 0.1% DMSO, both from Sigma-Aldrich, St. Louis, MO, USA), IL-1β (5.0 μg kg^{-1}, R&D Systems, Minneapolis, MN, USA), IL-1 receptor antagonist (IL-1ra, 90 or 900 μg kg^{-1}) (dissolved in 0.1% BSA/PBS, R&D Systems), 0.1% DMSO or 0.1% BSA/PBS (vehicle) were microinjected *in vivo* for 10 days. This dose of IL-1ra blocks depression-like behaviors in mice.[38,39]

For intra-hippocampal microinjection of adeno-associated virus (AAV) vectors containing the genes for caspase-1 (NCBI CCDS ID CCDS22798.1) or GFP alone (Genechem, Shanghai, China). Briefly, a total volume of 2.0 μl AAV vectors (1.0 μl per side) were delivered bilaterally into the CA1 region (AP = − 2.0 mm, ML = ± 1.2 mm, DV = − 2.0 mm) at 0.2 μl min^{-1} by glass capillaries with tip resistance of 5–10 MΩ in a stereotactic apparatus, followed by 10 min of rest to allow diffusion. Behavioral testing commenced 2 weeks following viral injection, then C57BL/6J mice were perfused transcardially, and serial brain sections were made. Fluorescence microscopy and western blotting could observe the effects of transfection *in vivo*.

Western blotting and other experiments. Western blotting was performed according to a protocol that is routinely used in our laboratory.[40] The surface expression of NMDARs and AMPARs was assayed by BS^3 crosslinking experiments. Gene expression, immunohistochemistry and ELISA were performed and analyzed as described in the Supplementary Information.

Statistical analysis

Data were expressed as the mean ± s.e.m. and analyzed using SPSS 18.0 software (SPSS, Chicago, IL, USA). In all behavioral assays, subjects were randomly assigned to several groups, and the experiments were performed blind to genotype or treatment.

The estimates of the necessary sample size(s) required were based on statistical criteria. Statistical analyses were performed using unpaired Student's *t*-tests, one or two-way analysis of variance, or repeated-measures analysis of variance, where appropriate. Significant effects in analysis of variances were followed by Bonferroni's *post hoc* multiple comparison tests. $P < 0.05$ was considered statistically significant.

RESULTS

Genetic ablation of caspase-1 in mice prevents chronic stress-induced depression-like behaviors

To identify the relevance of caspase-1 and different stressors, which are well recognized to mimic the precipitating factors of MDD, we first employed CSDS, CUMS animal model of depression. Following exposure to CSDS, C57BL/6J mice were separated into susceptible and resilient subpopulations (Figure 1b and Supplementary Figures 1 and 2a). Susceptible mice displayed a significant decrease in sucrose preference, body weight and memory impairment (Figure 1c and Supplementary Figures 2b–d) when compared with non-defeated control mice. As shown previously,[41] there was no change in serum corticosterone levels induced by CSDS (Supplementary Figure 2e), whereas both susceptible and resilient mice displayed a corticosterone response after a 6 min swim stress test following CSDS (Supplementary Figure 2f). Meanwhile, CUMS-treated mice also showed depression-like behavior, such as decreased sucrose preference and increased immobility time in TST (Supplementary Figures 2g and h).

Next, the levels of caspase-1 were examined under different stressors. We found that caspase-1 mRNA was significantly increased in the periphery blood mononuclear cell and hippocampus of susceptible mice induced by CSDS compared with control mice (Figure 1d), but remained unchanged in the striatum and amygdala (Supplementary Figure 2i). Moreover, we observed a significant negative correlation between the level of caspase-1 mRNA and SI ratio (Figure 1e). Furthermore, in contrast to control mice, susceptible mice displayed a significant increase in the level of the activated form of caspase-1 protein in the hippocampus (Figure 1f). However, caspase-1 protein levels were not increased in the thalamus or amygdala of susceptible mice (Supplementary Figure 2j), confirming the regional specificity of chronic stress-induced changes in caspase-1. Meanwhile, CUMS also significantly upregulated the level of cleaved form of capsase-1 protein in the hippocampus (Figure 1g).

Figure 1. Genetic ablation of caspase-1 in mice prevents chronic stress-induced depression-like behaviors. (**a**) The proposed schema depicting caspase-1-dependent IL-1β production and increased AMPA receptor internalization. (**b**) Horizontal scatter plot depicting the distribution of interaction ratios for control, susceptible, and resilient mice after CSDS. (**c**) Susceptible mice displayed anhedonia as measured by a reduction in 1% sucrose preference ($n = 15$ mice/ group, mean ± s.e.m., one-way ANOVA, Bonferroni's test, $^{***}P < 0.001$). (**d**) Caspase-1 mRNA level in the PBMC and hippocampus (Hip) after CSDS ($n = 5$–8 mice/group, mean ± s.e.m., one-way ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). (**e**) Correlation of caspase-1 mRNA level in the hippocampus with social interaction ratio after CSDS (Pearson correlation, $n = 11$–12 mice/group). (**f**) Representative immunoblots and quantification of caspase-1 protein expression in the hippocampus after CSDS ($n = 10$ mice/group, mean ± s.e.m., one-way ANOVA, Bonferroni's test, $^{**}P < 0.01$, $^{***}P < 0.001$). (**g**) CUMS increased the expression of caspase-1 protein in the hippocampus ($n = 10$–11 mice/group, mean ± s.e.m., Student's *t*-test, $^{***}P < 0.001$). (**h**) Social interaction times for WT, WT-CSDS, *Caspase-1*$^{-/-}$ and *Caspase-1*$^{-/-}$-CSDS mice, and *Caspase-1*$^{-/-}$ mice showed significant increase in social interaction after CSDS ($n = 10$ mice/group, mean ± s.e.m., repeated-measures ANOVA, Bonferroni's test, $^{***}P < 0.001$). (**i**) *Caspase-1*$^{-/-}$ mice blocked anhedonia as measured by a reduction in 1% sucrose preference after CSDS ($n = 10$ mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{***}P < 0.001$). (**j**) Effects of CSDS on the object recognition test ($n = 8$–10 mice/group, mean ± s.e.m., repeated-measures ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**k**) *Caspase-1*$^{-/-}$ mice reversed anhedonia as measured by a reduction in 1% sucrose preference after CRS ($n = 10$–11 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$, $^{***}P < 0.001$). (**l** and **m**) *Caspase-1*$^{-/-}$ mice prevented CRS-induced increase in immobility time in FST (**l**) and TST (**m**) when compared to WT-CRS mice ($n = 10$–11 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, analysis of variance; CUMS, chronic unpredictable mild strss; CRS, chronic restraint stress; CSDS, chronic social defeat stress; FST, forced swim test; IL, interleukin; PBMC, periphery blood mononuclear cell; TST, tail suspension test; WT, wild-type.

To determine the effect of caspase-1 on the depressive-like behavior induced by different stressors, the C57BL/6J mice with a genetic ablation of caspase-1 (*Caspase-1*$^{-/-}$) was used. Calcium/calmodulin-dependent protein kinase type II (CaMKII) signaling and the gross morphology of the hippocampus were unaffected by loss of caspase-1 (Supplementary Figures 3 and 4). Under non-stressed conditions, *Caspase-1*$^{-/-}$ mice, but not WT control mice, showed anxiolytic behavior in the OF and EPM tests (Supplementary Figure 5). When compared with WT mice, *caspase-1*$^{-/-}$ mice did not display differences in total fluid intake, sucrose consumption, vocalizing and jumping (Supplementary Figures 6a-c), but did exhibit increased locomotion (Supplementary Figure 6d).

We then compared *Caspase-1*$^{-/-}$ with WT mice in the CSDS model. Under non-defeated conditions, baseline SI and sucrose consumption of *Caspase-1*$^{-/-}$ mice were similar with those of WT littermates. Different from WT-CSDS mice, *Caspase-1*$^{-/-}$ mice exposed to CSDS showed increased body weight gain, SI and sucrose consumption (Figures 1h and i and Supplementary Figures 7a–d). Furthermore, impaired recognition memory was only observed with WT-CSDS mice (Figure 1j), which did not display a difference in the exploration time or inherent preference value of the novel object (Supplementary Figure 7e).

Next, *Caspase-1*$^{-/-}$ mice and their WT littermates were subjected to CRS. Compared with non-stressed control mice, WT-CRS mice displayed depression-like behaviors, including decreased sucrose consumption and increased immobility time in FST and TST (Figures 1k-m and Supplementary Figure 7f). Strikingly, the depression-like behaviors induced by CRS in WT mice were completely prevented in the *Caspase-1*$^{-/-}$ mice (Figures 1k-m and Supplementary Figure 7f). Moreover, serum corticosterone levels were higher in both CRS-treated groups when compared with controls (Supplementary Figure 7g), which was consistent with a previous study.[42]

Taken together, these findings confirm that *Caspase-1*$^{-/-}$ mice are resilient to chronic stress-induced depression-like behaviors.

Caspase-1 overexpression in the hippocampus increases depression- and anxiety-like behaviors

Next, we used a viral expression approach to examine whether the overexpression of caspase-1 in hippocampus is responsible for the behavioral effects of CSDS (Figure 2a). We stereotaxically injected an AAV serotype 1 vector encoding caspase-1 (AAV-caspase-1) into the hippocampus. The accuracy of the injection site was confirmed by immunofluorescence staining of GFP, which is co-expressed with caspase-1 by the AAV (Figure 2b and Supplementary Figure 8a). AAV-caspase-1 infusion resulted in significantly increased levels of caspase-1 and IL-1β protein (Figure 2c). We found that AAV-caspase-1 mice showed a significant decrease in SI and sucrose preference following subthreshold social defeat stress (Figures 2d and e and Supplementary Figures 8b-d) compared with control virus-injected mice (AAV-GFP). Further, AAV-caspase-1 mice displayed increased immobility time in the TST and FST compared with AAV-GFP mice (Figure 2f). The velocity or total distance traveled was not affected in AAV-caspase-1 mice (Supplementary Figure 8e), indicating that immobility was not likely due to motor defects. There is strong comorbidity between anxiety disorders and depression, and AAV-caspase-1 mice also displayed an anxiogenic phenotype. Compared with AAV-GFP mice, AAV-caspase-1 mice spent less time, distance traveled, and fewer visits in the central area of the OF (Figure 2g and Supplementary Figures 8e and g), and less time in the open arms and conversely more time in the closed arms in the EPM (Figure 2h and Supplementary Figures 8f and h). Together, these results suggest that caspase-1 overexpression in the hippocampus induces depression- and anxiety-like behaviors.

Genetic ablation of caspase-1 blocks CSDS-induced synaptic plasticity impairment in the hippocampus

Accumulating evidence suggests that chronic stress causes changes in the glutamatergic system, and dysregulation of glutamate signaling is increasingly considered to be a core feature of mood disorder.[1,12,13,43] To investigate the role of caspsase-1 in glutamate neurotransmission, we first examined the input/output curves in the CA1 region of hippocampal slices. The input/output curves were markedly reduced in the WT-CSDS group but remained unaffected in the *Caspase-1*$^{-/-}$-CSDS group (Figure 3a). No significant differences were observed in paired-pulse facilitation (Supplementary Figures 9a and b), suggesting a lack of gross change in presynaptic function in *Caspase-1*$^{-/-}$ mice. Compared with WT mice, *Caspase-1*$^{-/-}$ mice showed a normal induction and maintenance of LTP in Schaffer collateral-CA1 after CSDS (Figure 3b), but there was no change in NMDAR-dependent LTD (Supplementary Figures 9c and d). To determine if the suppression of CSDS on glutamatergic neurotransmission results from the reduced expression of glutamate receptors, we performed western blotting and surface receptor crosslinking with BS^3 assays. Following exposure to CSDS, *Caspase-1*$^{-/-}$ mice showed increased surface expression of GluA1 and GluA2 in the hippocampus compared with WT mice (Figure 3c). In addition, the total levels of AMPARs and NMDARs, and the surface expression of NMDARs, were unaltered in all groups (Figure 3d and Supplementary Figure 10). Furthermore, *Caspase-1*$^{-/-}$ mice completely blocked the reduction of PSD95 caused by CSDS in WT mice (Figure 3d), indicating CSDS-induced synaptic dysfunction is unique to excitatory glutamatergic neurons, but not GABAergic interneuron. Next, we measured mEPSCs in CA1 pyramidal neurons, which are mediated by AMPARs and reflected the individual synaptic responses from the quantal release of single glutamate vesicles. WT mice exposed to CSDS showed a greatly decreased mEPSC amplitude, which remained unchanged in the *Caspase-1*$^{-/-}$ mice upon exposure to CSDS. Furthermore, there were no effects of CSDS on mEPSC frequency in both mice (Figure 3e). In addition, CSDS significantly reduced the density of PSD95 immunofluorescence in WT but not in *Caspase-1*$^{-/-}$ mice (Figure 3f and Supplementary Figure 11), consistent with the observation that hippocampal neurons were not lost in these mice after chronic stress.[44] These results suggest that the deletion of caspase-1 increases the resilience to chronic stress by maintaining normal postsynaptic glutamate neurotransmission.

Interestingly, only susceptible mice showed a significant increase in serum IL-1β compared with control mice (Supplementary Figure 12a). It is well established that IL-1β is a downstream target of caspase-1, and that IL-1β maturation depends on caspase-1 activation.[34] Thus, we examined the effect of caspsase-1 loss on IL-1β levels. As shown by ELISA, CSDS increased the level of serum IL-1β in WT mice, but not in *Caspase-1*$^{-/-}$ mice (Figure 3g). Furthermore, ablation of caspase-1 prevented the CSDS-induced elevation of IL-1β mRNA and protein levels in the hippocampus (Figures 3h and i).

Inflammasome cleaves pro-caspase-1 into mature caspase-1, which subsequently mediates the maturation of IL-1β. Therefore, we tested whether inflammasomes modulate CSDS-induced depression. CSDS exposure increased the mRNA level of nucleotide-binding oligomerization domain-like receptor pyrin domain-containing protein 3 (NLRP3) in WT and *Caspase-1*$^{-/-}$ mice, but not of NLRP1 and absent in melanoma 2 mRNA (Supplementary Figures 12b-d). Interestingly, CSDS failed to increase the expression of apoptosis-associated speck-like protein containing a CARD (ASC) mRNA in *Caspase-1*$^{-/-}$ mice compared with WT mice (Supplementary Figure 12e). These results indicate that CSDS activates the NLRP3 inflammasome, leading to the increased release of caspase-1-dependent IL-1β. IL-1β can suppress BDNF-dependent synaptic plasticity via the p38 mitogen-activated

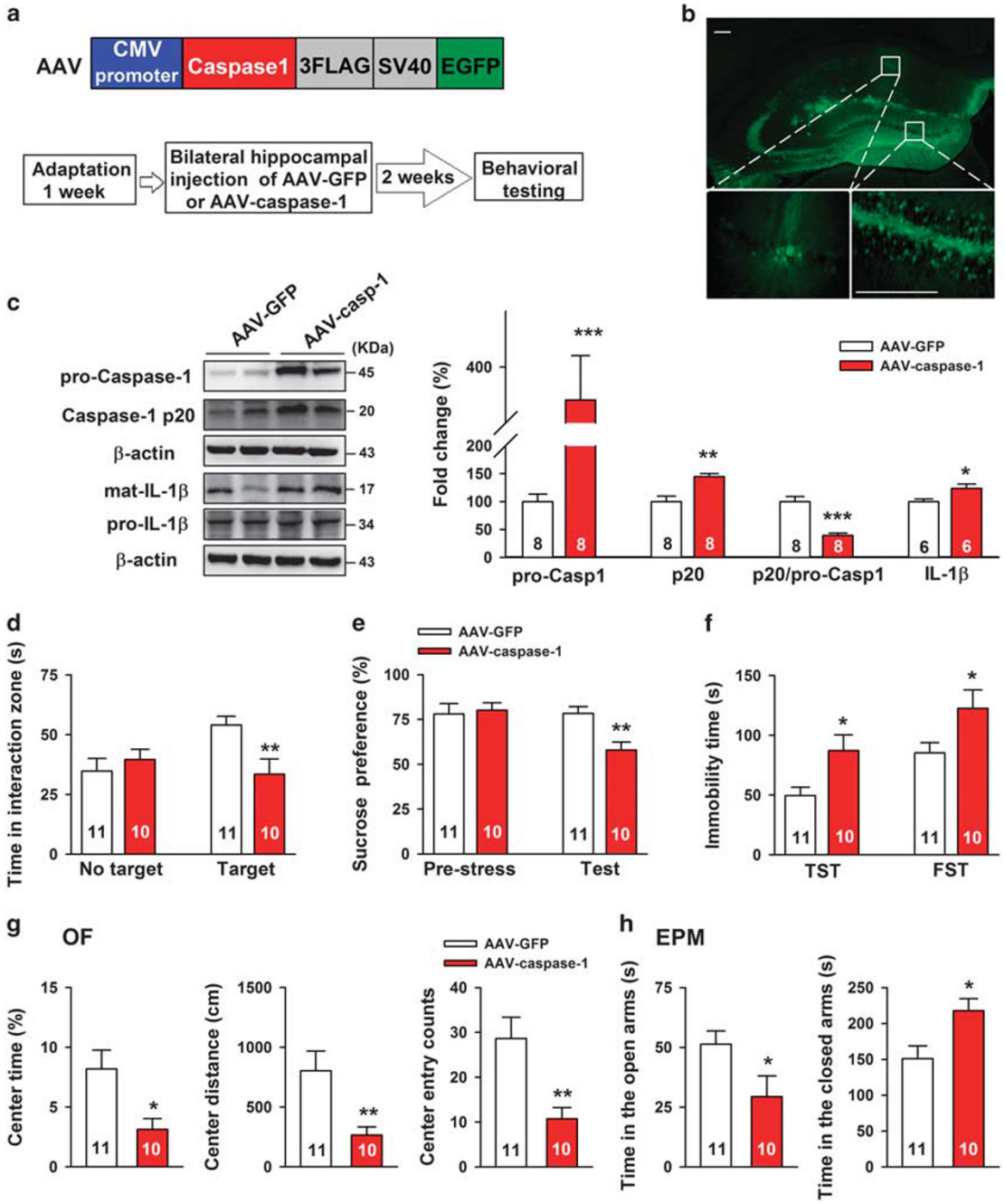


Figure 2. Overexpression of caspase-1 in the hippocampus increases depression- and anxiety-like behaviors. (**a**, top) Schematic representation of construct showing mouse caspase-1 subcloned into an AAV plasmid under transcriptional regulation of the CMV promoter (AAV-caspase-1-3FLAG-SV40-EYFP). AAV-3FLAG-SV40-EYFP plasmid without encoding caspase-1 served as the control. Bottom, the timeline of experimental procedure. (**b**) Representative photomicrographs of injection sites in the hippocampus. Scale bars, 200 μm. (**c**) Representative western blotting of hippocampal pro-Caspase-1, Caspase-1 p20, Caspase-1 p20/pro-Caspase-1, IL-1β after subthreshold social defeat stress (left). Mean (±s.e.m.) fold change in protein from hippocampus microdissections from AAV-GFP or AAV-caspase-1-injected mice (right) ($n = 6$–8 mice/group, mean ± s.e.m., Student's *t*-test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). (**d** and **e**) AAV-caspase-1-injected mice increased social avoidance (**d**) and decreased sucrose preference (**e**) after subthreshold social stress when compared with their respective AAV-GFP ($n = 10$–11 mice/group, mean ± s.e.m., repeated-measures ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**f**) Summary of the TST and FST in AAV-GFP and AAV-caspase-1-injected mice. Graphs showed an increase in time spent immobile in AAV-caspase-1-injected mice ($n = 10$–11 mice/group, mean ± s.e.m., Student's *t*-test, $^*P < 0.05$). (**g** and **h**) AAV-caspase-1-injected mice displayed an anxiogenic phenotype in OF (**g**) and EPM (**h**) when compared with their respective AAV-GFP ($n = 10$–11 mice/group, mean ± s.e.m., Student's *t*-test, $^*P < 0.05$, $^{**}P < 0.01$). AAV, adeno-associated virus; ANOVA, analysis of variance; CMV, cytomegalovirus; EPM, elevated plus maze; FST, forced swim test; GFP, green fluorescent protein; OF, open-field test; TST, tail suspension test.

protein kinase (MAPK) pathway.[45] Further, our previous study showed that CSDS significantly reduced the expression of BDNF in the hippocampus of susceptible mice.[46] Thus, we evaluated whether CSDS could change the levels of phosphorylated p38 MAPK and BDNF in *Caspase-1*$^{-/-}$ mice. As predicted, phosphorylation of p38 MAPK was significantly elevated and BDNF was decreased in the WT-CSDS group; however, their levels were unaltered in the *Caspase-1*$^{-/-}$-CSDS group (Figure 3i). Taken

together, these results suggest that caspase-1-mediated neuroinflammation affects glutamatergic neurotransmission, leading to CSDS-induced depression-like behaviors.

Pharmacological inhibition of the caspase-1 signaling pathway prevents CSDS-induced depression-like behaviors and synaptic impairment

Next, we examined the effect of the caspase-1-specific inhibitor AC-YVAD-CMK on depression-like behaviors. A single intracerebroventricular (i.c.v.) microinfusion of AC-YVAD-CMK significantly decreased immobility time of mice in a dose-dependent manner in FST as well as TST without affecting locomotion (Supplementary Figures 13a and b). Thus, acute caspase-1 inhibitor treatment produces antidepressant-like effects in mice. Further, we found that WT-CSDS mice repeatedly pretreated with AC-YVAD-CMK exhibited normal SI and sucrose preference compared to control mice (Figure 4a and Supplementary Figures 13c–e), confirming the effect of AV-YVAD-CMK on CSDS-induced depressive behaviors. Moreover, AC-YVAD-CMK treatment also prevented the increase in caspase-1 and IL-1β expression (Figure 4b), as well as the

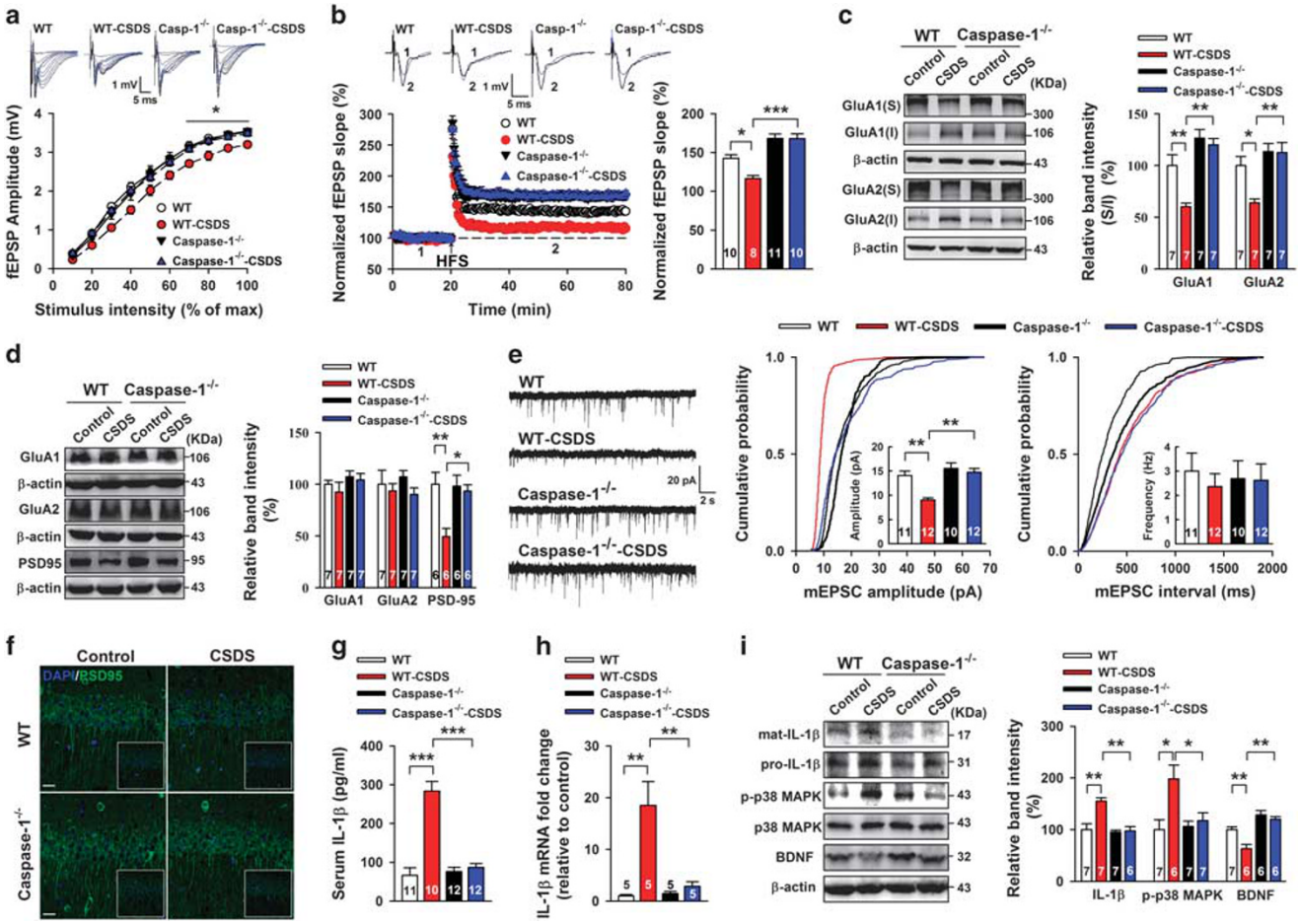


Figure 3. Genetic ablation of caspase-1 blocks CSDS-induced synaptic plasticity impairment in the hippocampus. (**a**) Input–output curves illustrating the relationship between the magnitudes of stimulation and evoked response for fEPSPs recorded in hippocampal slices from WT, WT-CSDS, *Caspase-1*$^{-/-}$ and *Caspase-1*$^{-/-}$-CSDS groups. Insets are typical superimposed fEPSPs recorded by increasing stimulation intensity ($n = 10$ slices from four to seven mice per group, mean ± s.e.m., repeated-measures ANOVA, Bonferroni's test, $^{*}P < 0.05$). (**b**, left) time-course of LTP induced by HFS in hippocampal slices from different groups. Insets are superimposed fEPSPs recorded for each condition before (1) and 60 min after the application of HFS (2). (right) The histogram showing LTP magnitude averaged from the last 15 min of recordings from different groups ($n = 8$–11 slices from four to seven mice per group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). (**c**) Representative immunoblots and quantification of surface expression of GluA1 and GluA2 in the hippocampus from different groups ($n = 7$ mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). (**d**) Representative immunoblots and quantification of total expression of hippocampal GluA1, GluA2 and PSD95 proteins from different groups ($n = 6$–7 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). (**e**, left) Representative whole-cell voltage-clamp traces of AMPAR-mediated mEPSC in the hippocampal pyramidal neurons. Scale is depicted on the middle right. (right) Mean ± s.e.m. amplitude and frequency of AMPAR-mediated mEPSC from different groups ($n = 10$–12 cells from five to six mice per group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**f**) Immunofluorescence of PSD95 in CA1 pyramidal neurons. Scale bars, 100 μm. (**g**) The levels of IL-1β in the serum were determined by ELISA in the different groups ($n = 10$–12 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{***}P < 0.001$). (**h**) Analysis of IL-1β mRNA expression in the hippocampus by qRT-PCR. ($n = 5$ mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**i**) Representative immunoblots and quantification of hippocampal IL-1β, p-p38 MAPK and BDNF proteins ($n = 6$–7 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANOVA, analysis of variance; BNDF, brain-derived neurotrophic factor; CSDS, chronic social defeat stress; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency; stimulation; I, intracellular; IL, interleukin; LTP, long-term potentiation; mEPSC, miniature excitatory postsynaptic current; S, surface; qRT-PCR, quantitative PCR with reverse transcription; WT, wild-type.

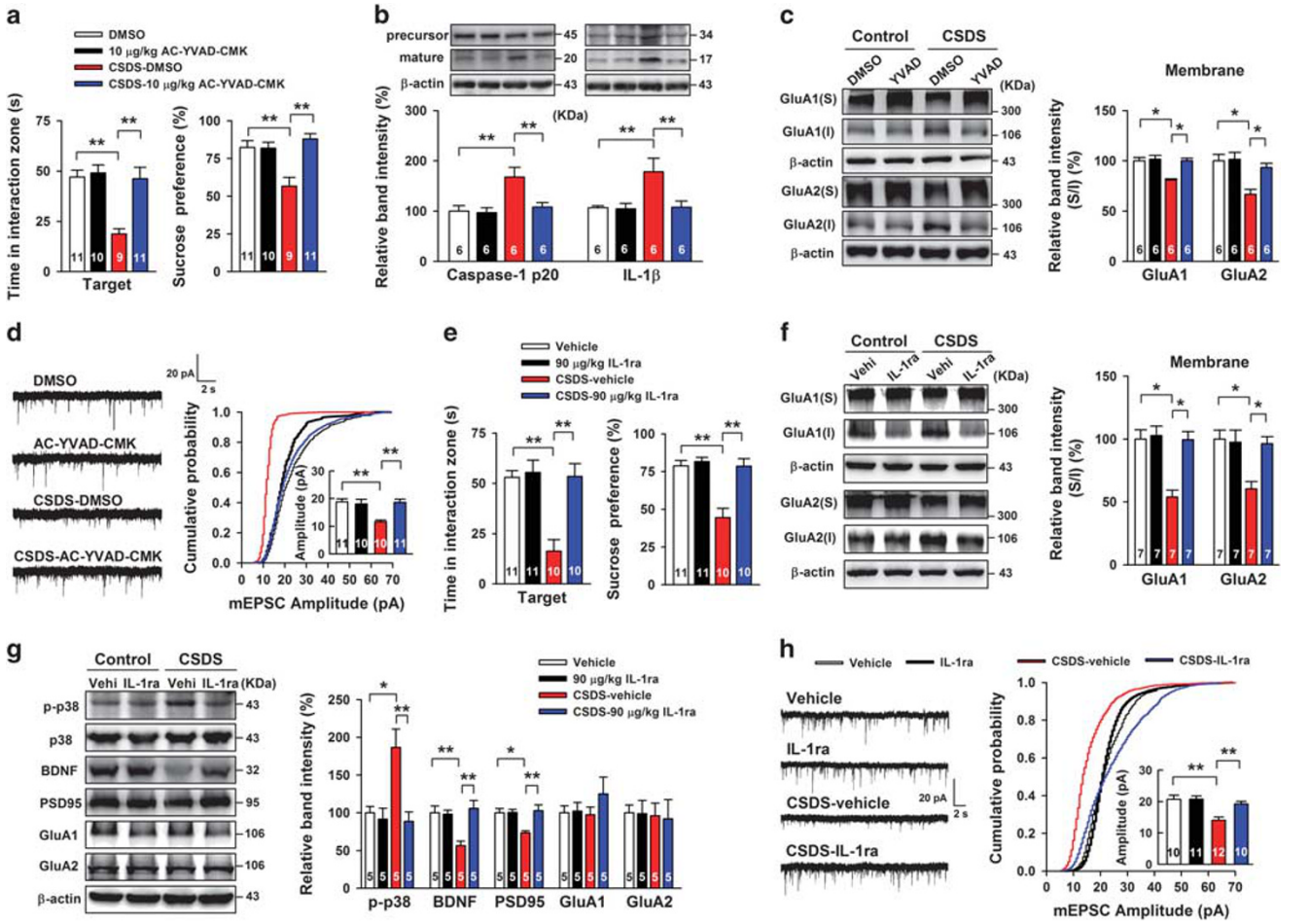


Figure 4. Pharmacological inhibition of caspase-1 signaling pathway prevents CSDS-induced depression-like behaviors and synaptic plasticity impairment. (**a**) I.c.v. infusion of AC-YVAD-CMK 30 min before daily social defeat for 10 days rendered the mice more resilient to CSDS in social interaction (left) and sucrose preference (right) test ($n = 9$–11 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**b**) Representative immunoblots and quantification of hippocampal caspase-1 and IL-1β proteins from DMSO, 10 μg kg^{-1} AC-YVAD-CMK, CSDS-DMSO and CSDS-10 μg kg^{-1} AC-YVAD-CMK mice ($n = 6$ mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**c**) Representative immunoblots and quantification of surface expression of GluA1 and GluA2 in the hippocampus from different groups ($n = 6$ mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$). S, surface; I, intracellular. (**d**, left) Representative whole-cell voltage-clamp traces of AMPAR-mediated mEPSC in the hippocampal pyramidal neurons. Scale is depicted on the middle right. (right) Mean ± s.e.m. amplitude of AMPAR-mediated mEPSC from different groups ($n = 10$–11 cells from three to four mice per group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**e**) Continuous administration of IL-1ra during CSDS improved social interaction (left) and sucrose preference (right) reduction in stressed mice ($n = 10$–11 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). (**f**) Representative immunoblots and quantification of surface expression of GluA1 and GluA2 in the hippocampus ($n = 7$ mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$). (**g**) Representative immunoblots and quantification of hippocampal p-p38 MAPK, BDNF, PSD95, total GluA1 and GluA2 proteins from Vehicle, IL-1ra, CSDS-vehicle and CSDS-IL-1ra mice, and the reduction in the hippocampus of stressed mice were significantly prevented by IL-1ra administration ($n = 5$ mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). (**h**, left) Representative whole-cell voltage-clamp traces of AMPAR-mediated mEPSC in the hippocampal pyramidal neurons. Scale is depicted on the middle right. (right) Mean ± s.e.m. amplitude of AMPAR-mediated mEPSC from different groups ($n = 10$–12 cells from four to six mice per group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{**}P < 0.01$). AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CSDS, chronic social defeat stress; DMSO, dimethylsulphoxide; I, intracellular; IL, interleukin; S, surface.

reduction of surface levels of GluA1 and GluA2, in response to CSDS (Figure 4c). There was no significant difference in the total levels of GluA1 and GluA2 (Supplementary Figure 13f). Furthermore, we observed a decrease in the amplitude of mEPSC in CSDS-treated mice, but not in CSDS-AC-YVAD-CMK-treated mice, and the frequency of mEPSC remained unchanged (Figure 4d and Supplementary Figure 13g), confirming that pharmacological inhibition of caspase-1 prevents CSDS-induced depression-like behaviors and the impairment of synaptic plasticity.

To further confirm a direct role of caspase-1-mediated IL-1β signaling pathway in CSDS-induced depression-like behaviors, we next investigated whether the blockade of IL-1β could prevent depression in WT-CSDS mice. Although a single i.c.v. infusion of recombinant mouse IL-1ra had no effect on sucrose preference, locomotion and anxiety behaviors (Supplementary Figures 14a–c), repeated i.c.v. infusions of IL-1ra prevented CSDS-induced social avoidance and sucrose preference reduction in a dose-dependent manner (Figure 4e and Supplementary Figures 14d-f). Consistent with these behavioral results, IL-1ra also blocked the decrease in surface expression of GluA1 and GluA2 caused by CSDS, whereas the total proteins levels remained unchanged (Figures 4f and g). Furthermore, IL-1ra not only reduced the expression of

Figure 5. Chronic IL-1β exposure induces depression and anxiety-like behaviors in *Caspase-1*$^{-/-}$ mice. (**a**) Timeline of experimental procedure. D, day. (**b**) The body weight was measured during the experimental procedure, and there were no changes in WT-vehicle, WT-IL-1β, *Caspase-1*$^{-/-}$-vehicle and *Caspase-1*$^{-/-}$-IL-1β ($n = 10$–12 mice/group, mean ± s.e.m., repeated-measures ANOVA, Bonferroni's test). (**c** and **d**) WT and *Caspase-1*$^{-/-}$ mice displayed decreased social interaction following IL-1β administration for 10 days when compared to their respective vehicle-injected WT and *Caspase-1*$^{-/-}$ controls ($n = 10$–12 mice/group, mean ± s.e.m., repeated-measures ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). (**e**) Chronic i.c.v. infusion of IL-1β induced anhedonia as measured by 1% sucrose preference. Both WT and *Caspase-1*$^{-/-}$ mice that received repeated IL-1β infusion showed significant reduction on days 10 and 12 ($n = 10$ mice/group, mean ± s.e.m., repeated-measures ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). (**f** and **g**) Chronic i.c.v. infusion of IL-1β in both WT and *Caspase-1*$^{-/-}$ mice significantly increased anxiety in OF (**f**) and EPM (**g**) when compared to their controls ($n = 10$–12 mice/group, mean ± s.e.m., two-way ANOVA, Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$). ANOVA, analysis of variance; EPM, elevated plus maze; IL, interleukin; OF, open-field test; WT, wild-type.

phosphorylated p38 MAPK and increased the levels of BDNF and PSD95 in CSDS-treated mice (Figure 4g), but also prevented the reduction of mEPSC amplitude caused by CSDS, with no effect on mEPSC frequency (Figure 4h and Supplementary Figure 14g). Taken together, these results further suggest that the inhibition of the caspase-1 signaling pathway prevents CSDS-induced depression-like behaviors by stabilizing the surface expression of AMPARs in the hippocampus.

Chronic IL-1β exposure induces depression and anxiety-like behaviors in *Caspase-1*$^{-/-}$ mice

Previous studies demonstrate that chronic subcutaneous administration of IL-1β can mimic the effects of CUMS-induced depression.[28] Thus, we investigated whether chronic central administration of IL-1β could block the antidepressant effect of caspase-1 deletion. Following IL-1β administration for 10 days (Figure 5a), there were no changes in the body weights of either WT or *Caspase-1*$^{-/-}$ mice (Figure 5b). Interestingly, chronic i.c.v. infusion of IL-1β significantly decreased SI in both WT and *Caspase-1*$^{-/-}$ mice on days 6 and 11, whereas no difference in SI was observed on day 1 (Figures 5c and d and Supplementary Figure 15a). Consistent with these results, both WT and *Caspase-1*$^{-/-}$ mice exhibited a significant decrease in sucrose preference on days 10 and 12 (Figure 5e and Supplementary Figure 15b), suggesting that caspase-1-dependent IL-1β production may mediate chronic stress-induced depression-like behaviors.

Furthermore, chronic i.c.v. infusion of IL-1β reduced the number of entries and time spent in the central area in the OF (Figure 5f), shortened the time spent in the open arms and prolonged the time spent in the closed arms in the EPM (Figure 5g). However, there was no effect observed on locomotion, total distance traveled, total arm entries into any arms or open arm entry counts (Supplementary Figures 15c-d), indicating that IL-1β is essential for caspase-1-mediated depression- and anxiety-like behaviors.

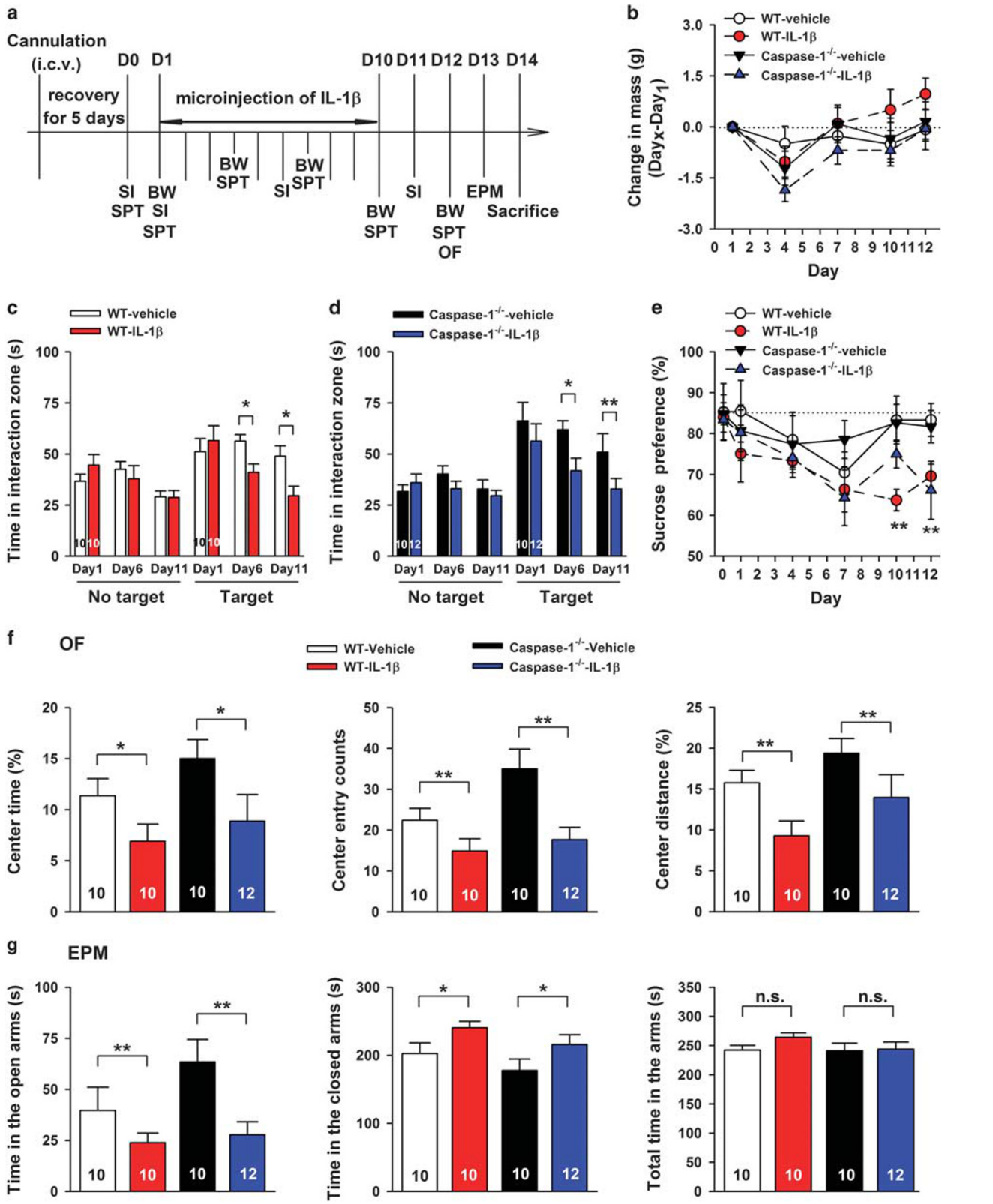
DISCUSSION

Caspase-1 has usually been recognized as an inflammatory caspase that is involved in the general response to inflammatory stimuli. In this study, to the best of our knowledge, we have provided direct evidence that, in addition to the classical effects on cytokine maturation and the center of many cell responses to inflammation, caspase-1-mediated surface stability of AMPAR in the hippocampus was essential for the synaptic plasticity impairment and depression-like behavior in response to chronic stress. We showed that loss of caspase-1 caused antidepressant-like effects in mice, and conversely, that overexpression of caspase-1 in the hippocampus of WT mice induced depression- and anxiety-like behaviors. Pharmacological inhibition of the caspase-1-IL-1β signaling pathway protected against CSDS-induced depression. Importantly, we found that inhibition of either caspase-1 or IL-1β could prevent the decrease in surface expression of AMPARs and AMPAR-mediated mEPSC caused by CSDS. Further, caspase-1-activated IL-1β signaling, followed by transcriptional downregulation of BDNF, eventually contributed to impaired synaptic plasticity (Supplementary Figure 16). Our results further provide a rigorous analysis of the convergence of the inflammation and glutamate hypotheses of depression.

We developed an approach to investigate the critical role of caspase-1 in the development of depression. Briefly, we divided the mice exposed to various stressful stimuli, and enabled further establishment of several lines of evidence supporting a critical role of caspase-1 in the active and adaptive development of chronic stress-induced depression. We found that the level of caspase-1 was increased in the periphery after social defeat in susceptible mice. Meanwhile, we demonstrated that *Caspase-1*$^{-/-}$ mice were resilience to chronic stress without changes in the gross neuroanatomy and morphological structure of the hippocampus. Furthermore, a caspase-1-specific inhibitor displayed an antidepressant effect. Interestingly, glucocorticoid levels are dramatically elevated in depressed patients and animals;[48] however, we found that corticosterone was not involved in the caspase-1-mediated depression induced by CSDS. Taken together, these results indicate that caspase-1 has an essential role in the depression, and the level of caspase-1 in the peripheral blood mononuclear cells may be a reliable biomarker for depression.

Caspase-1 regulates food intake and memory through affecting IL-1β processing.[49,50] Moreover, *Caspase-1*$^{-/-}$ mice showed impaired processing of pro-IL-1β and reduced secretion of IL-1β after lipopolysaccharide stimulation.[34] Thus, the maturation of IL-1β is one of the main mechanisms whereby caspase-1 regulates behavioral alteration. Consistent with these findings, our results showed that chronic infusion of IL-1β not only abolished the antidepressant-like effects in *Caspase-1*$^{-/-}$ mice, but also increased anxiety in both WT and *Caspase-1*$^{-/-}$ mice. Importantly, chronic infusion of specific caspase-1 inhibitor AC-YVAD-CMK prevented CSDS-induced depression-like behaviors and synaptic dysfunction of susceptible mice via suppression of IL-1β expression, as confirmed by administration of IL-1ra, indicating that activated caspase-1 results in increased depression- and anxiety-like behaviors in an IL-1β-dependent manner.

Caspase-5, a component of the NLRP1 inflammasome, mediates the generation of active IL-1β.[51,52] It has been shown that chronic glucocorticoids exposure increases hippocampal caspase-5.[53] However, our results showed that CSDS robustly increased the level of NLRP3 mRNA, the major contributor to caspase-1 activation and chronic stress-induced depression,[11,54,55] without effect on NLRP1 mRNA, suggesting that caspase-5 may not be required to elicit depression-like behaviors. Further, NLRP3 activates caspase-1 in an ASC-dependent manner. ASC levels are significantly elevated in periphery blood mononuclear cells from depressed patients and in the hippocampus of mice exposure to chronic glucocorticoids,[53,56] However, our results showed that ASC mRNA was unaltered in *Caspase-1*$^{-/-}$ mice after CSDS, indicating that ASC may be not required for CSDS-induced caspase-1 activation and depression. Notably, recent work has demonstrated that deletion of any gene in the NLRP3-caspase-1-IL-1β axis reduces anxiety and depression-like behavior by EPM, OF and dark–light-box test.[8,47,55,57] We will further confirm the effects of caspase-1 inhibition by using dark–light-box and learned helplessness paradigm.



Recent studies suggest that the changes in excitatory synapses are a fundamental pathophysiology of depression, and that restoration of synaptic plasticity impairment is critical to the relief of depression.[12,18,19] Recent studies reveal that genetic ablation of the vesicular transporter 1 (VGLUT1) not only mimic exclusively mood disorder but also induce abnormalities associated with schizophrenia through regulation of presynaptic glutamatergic neurotransmission.[58–60] Moreover, stress-induced depressive behaviors are associated with altered expression of VGLUT1.[61,62] Indeed, our results not only support a glutamate hypothesis of

depression, but also are consistent with previous reports implicating that chronic stress impairs hippocampal dependent cognition.[63] We postulate that the dysfunction in glutamatergic neurotransmission is critical for the memory deficits, and the causal relationship is mediated by AMPARs, which is one of the major receptors involved in synaptic plasticity, learning and memory.[64] This was supported by our results that CSDS inhibited hippocampal LTP by the activation of caspase-1 and the reduction of glutamatergic neurotransmission, including AMPAR-mediated mEPSC and surface expression of AMPARs. Moreover, the results that CSDS did not change the paired-pulse facilitation and the frequency of mEPSC but decreased the amplitude of mEPSC indicate that postsynaptic rather than presynaptic mechanisms may underlie the antidepressive effect of caspase-1 deficiency.

In addition to glutamatergic systems, the dysregulation of GABAergic system has been observed in the animal exposure to chronic stress. Hu and Filipovic *et al*. reported that chronic stress impaired GABAergic transmission by decreasing the parvalbumin (PV)-immunoreactive cells in the CA1, CA3 and dentate gyrus region of the hippocampus, but the expression of chaperone-inducible heat-shock protein 70 was unaffected in chronic stress alone.[65,66] Meanwhile, blockade of caspase-1 reverses the age-dependent decrease in hippocampal neurogenesis.[67] Further studies also need to investigate the role of caspase-1 deficiency in hippocampal neurogenesis and PV expression in MDD.

Importantly, the suppressive effect of loss of caspase-1 on synaptic plasticity depends on IL-1β production. Previous studies demonstrate that IL-1β impairs synaptic plasticity by activating JNK, nuclear factor-κB and caspase-1,[68,69] and increases NMDAR function through activation of Src family kinases.[70] However, we found that IL-1β activation selectively inhibited the surface expression of AMPARs, without an effect on the NMDARs, which may be mediated by stimulation of the IL-1 type I receptor (IL-1R1) and p38 MAPK-dependent signaling pathway. Moreover, these effects were not observed in *Caspase-1*$^{-/-}$ mice, which was consistent with the selective negative regulation of caspase-1 on memory and AMPAR-mediated synaptic transmission.[25,68] NLRP3 is expressed in microglia,[71] and therefore it is likely that CSDS activates microglia, and further causes caspase-1-generated IL-1β-mediated neuroinflammation and dysregulation of glutamatergic neurotransmission through neural microcircuits. However, some limitations should be considered when interpreting these results. Briefly, we still cannot exclude the possibility that the effects of chronic stress on caspase-1 are due to the activation of microglia rather than neurons, thereby directly regulating AMPAR trafficking.

Previous studies have shown a protective effect of probiotics on hippocampal dependent memory and synaptic plasticity.[72,73] Interestingly, genetic deficiency or pharmacological inhibition of caspase-1 prevents CRS-induced depressive-like behaviors through modulating gut microbiota composition.[8] Moreover, gut microbiota via inflammasome signaling may alter brain function. Our results provide direct evidence that caspase-1-mediated neuroinflammation affects glutamatergic neurotransmission by regulating the stability of surface AMPARs, which then further alters depressive behaviors. We proposed that direct modulation of caspase-1 may offer new opportunities to mitigate depressive disorders, via the regulation of glutamatergic neurotransmission in central nervous system and the interface between stress and gut microbiota in peripheral tissues, which provides further understanding of the 'inflammasome hypothesis' of depression.

In summary, the present study identifies a critical role for caspase-1/IL-1β signaling pathway-mediated surface stability of AMPAR in the hippocampus is essential for the impairment of synaptic plasticity and depression-like behavior in response to chronic stress, which is very different from the classical proinflammatory action of caspase-1/IL-1β signaling pathway. Our study couples the inflammatory caspase-1 signaling pathway with depression-like behavior via modulation of AMPAR trafficking and synaptic plasticity. Taken together, these results suggest that caspase-1 is critical for the development of depression-like behaviors, and highlights caspase-1 as a potential novel therapeutic target for the treatment of mood disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by grants from the National Basic Research Program of China (the 973 Program, No. 2013CB531303 to JGC; No. 2014CB744601 to FW), The National Natural Scientific Foundation of China (NSFC, No. 81473198, 81673414 to JGC; No. 81471377 to FW), The Innovation Group of Natural Science Fund of Hubei Province (No. 2015CFA020), PCSIRT (No. IRT13016) and the Fundamental Research Funds for the Central Universities, HUST (2015ZDTD045) to JGC.

AUTHOR CONTRIBUTIONS

MXL, GH, FW and JGC conceived and designed the project and wrote the manuscript, MXL, HLZ, JGH, JH, LZ, XW, WW, HYZ performed the behavioral experiments. MXL and YL performed the electrophysiology. MXL and HLZ performed the immunohistochemistry. MXL performed the biochemical measurements.

REFERENCES

1 Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat Med* 2016; **22**: 238–249.
2 Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature* 2008; **455**: 894–902.
3 McKim DB, Niraula A, Tarr AJ, Wohleb ES, Sheridan JF, Godbout JP. Neuroinflammatory dynamics underlie memory impairments after repeated social defeat. *J Neurosci* 2016; **36**: 2590–2604.
4 Setiawan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G *et al.* Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry* 2015; **72**: 268–275.
5 Janelidze S, Mattei D, Westrin A, Traskman-Bendz L, Brundin L. Cytokine levels in the blood may distinguish suicide attempters from depressed patients. *Brain Behav Immun* 2011; **25**: 335–339.
6 Miao EA, Rajan JV, Aderem A. Caspase-1-induced pyroptotic cell death. *Immunol Rev* 2011; **243**: 206–214.
7 Alcocer-Gomez E, de Miguel M, Casas-Barquero N, Nunez-Vasco J, Sanchez-Alcazar JA, Fernandez-Rodriguez A *et al.* NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. *Brain Behav Immun* 2014; **36**: 111–117.
8 Wong ML, Inserra A, Lewis MD, Mastronardi CA, Leong L, Choo J *et al.* Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Mol Psychiatry* 2016; **21**: 797–805.
9 Lawson MA, McCusker RH, Kelley KW. Interleukin-1 beta converting enzyme is necessary for development of depression-like behavior following intracerebroventricular administration of lipopolysaccharide to mice. *J Neuroinflammation* 2013; **10**: 54.
10 Zhang Y, Liu L, Peng YL, Liu YZ, Wu TY, Shen XL *et al.* Involvement of inflammasome activation in lipopolysaccharide-induced mice depressive-like behaviors. *CNS Neurosci Ther* 2014; **20**: 119–124.
11 Zhang Y, Liu L, Liu YZ, Shen XL, Wu TY, Zhang T *et al.* NLRP3 inflammasome mediates chronic mild stress-induced depression in mice via neuroinflammation. *Int J Neuropsychopharmacol* 2015; **18**: pii: pyv006.
12 Cai X, Kallarackal AJ, Kvarta MD, Goluskin S, Gaylor K, Bailey AM *et al.* Local potentiation of excitatory synapses by serotonin and its alteration in rodent models of depression. *Nat Neurosci* 2013; **16**: 464–472.
13 Thompson SM, Kallarackal AJ, Kvarta MD, Van Dyke AM, LeGates TA, Cai X. An excitatory synapse hypothesis of depression. *Trends Neurosci* 2015; **38**: 279–294.
14 Sanacora G, Treccani G, Popoli M. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology* 2012; **62**: 63–77.

15 Maes M, Verkerk R, Vandoolaeghe E, Lin A, Scharpe S. Serum levels of excitatory amino acids, serine, glycine, histidine, threonine, taurine, alanine and arginine in treatment-resistant depression: modulation by treatment with antidepressants and prediction of clinical responsivity. *Acta Psychiatr Scand* 1998; **97**: 302–308.
16 Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP *et al.* Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proc Natl Acad Sci USA* 2005; **102**: 15653–15658.
17 Lim BK, Huang KW, Grueter BA, Rothwell PE, Malenka RC. Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens. *Nature* 2012; **487**: 183–189.
18 Yuen EY, Wei J, Liu W, Zhong P, Li X, Yan Z. Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron* 2012; **73**: 962–977.
19 Gomez-Galan M, De Bundel D, Van Eeckhaut A, Smolders I, Lindskog M. Dysfunctional astrocytic regulation of glutamate transmission in a rat model of depression. *Mol Psychiatry* 2013; **18**: 582–594.
20 Maldonado-Irizarry CS, Swanson CJ, Kelley AE. Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *J Neurosci* 1995; **15**: 6779–6788.
21 Kallarackal AJ, Kvarta MD, Cammarata E, Jaberi L, Cai X, Bailey AM *et al.* Chronic stress induces a selective decrease in AMPA receptor-mediated synaptic excitation at hippocampal temporoammonic-CA1 synapses. *J Neurosci* 2013; **33**: 15669–15674.
22 Akinfiresoye L, Tizabi Y. Antidepressant effects of AMPA and ketamine combination: role of hippocampal BDNF, synapsin, and mTOR. *Psychopharmacology (Berl)* 2013; **230**: 291–298.
23 Chourbaji S, Vogt MA, Fumagalli F, Sohr R, Frasca A, Brandwein C *et al.* AMPA receptor subunit 1 (GluR-A) knockout mice model the glutamate hypothesis of depression. *FASEB J* 2008; **22**: 3129–3134.
24 Vogt MA, Elkin H, Pfeiffer N, Sprengel R, Gass P, Inta D. Impact of adolescent GluA1 AMPA receptor ablation in forebrain excitatory neurons on behavioural correlates of mood disorders. *Eur Arch Psychiatry Clin Neurosci* 2014; **264**: 625–629.
25 Lu C, Wang Y, Furukawa K, Fu W, Ouyang X, Mattson MP. Evidence that caspase-1 is a negative regulator of AMPA receptor-mediated long-term potentiation at hippocampal synapses. *J Neurochem* 2006; **97**: 1104–1110.
26 Bellinger FP, Madamba S, Siggins GR. Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Res* 1993; **628**: 227–234.
27 Lai AY, Swayze RD, El-Husseini A, Song C. Interleukin-1 beta modulates AMPA receptor expression and phosphorylation in hippocampal neurons. *J Neuroimmunol* 2006; **175**: 97–106.
28 Goshen I, Kreisel T, Ben-Menachem-Zidon O, Licht T, Weidenfeld J, Ben-Hur T *et al.* Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry* 2008; **13**: 717–728.
29 Rossi S, Sacchetti L, Napolitano F, De Chiara V, Motta C, Studer V *et al.* Interleukin-1beta causes anxiety by interacting with the endocannabinoid system. *J Neurosci* 2012; **32**: 13896–13905.
30 Patel NA, Romero AA, Zadina JE, Chang SL. Chronic exposure to morphine attenuates expression of interleukin-1 beta in the rat hippocampus. *Brain Res* 1996; **712**: 340–344.
31 Maeng S, Zarate CA Jr., Du J, Schloesser RJ, McCammon J, Chen G *et al.* Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* 2008; **63**: 349–352.
32 Reus GZ, Nacif MP, Abelaira HM, Tomaz DB, dos Santos MA, Carlessi AS *et al.* Ketamine ameliorates depressive-like behaviors and immune alterations in adult rats following maternal deprivation. *Neurosci Lett* 2015; **584**: 83–87.
33 Liu M, Li J, Dai P, Zhao F, Zheng G, Jing J *et al.* Microglia activation regulates GluR1 phosphorylation in chronic unpredictable stress-induced cognitive dysfunction. *Stress* 2015; **18**: 96–106.
34 Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C *et al.* Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. *Cell* 1995; **80**: 401–411.
35 Golden SA, Covington HE 3rd, Berton O, Russo SJ. A standardized protocol for repeated social defeat stress in mice. *Nat Protoc* 2011; **6**: 1183–1191.
36 Wang W, Sun D, Pan B, Roberts CJ, Sun X, Hillard CJ *et al.* Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. *Neuropsychopharmacology* 2010; **35**: 2249–2261.
37 Mao LM, Wang W, Chu XP, Zhang GC, Liu XY, Yang YJ *et al.* Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. *Nat Neurosci* 2009; **12**: 602–610.
38 Bluthe RM, Dantzer R, Kelley KW. Central mediation of the effects of interleukin-1 on social exploration and body weight in mice. *Psychoneuroendocrinology* 1997; **22**: 1–11.
39 Norman GJ, Karelina K, Zhang N, Walton JC, Morris JS, Devries AC. Stress and IL-1beta contribute to the development of depressive-like behavior following peripheral nerve injury. *Mol Psychiatry* 2010; **15**: 404–414.
40 Lu HF, Wu PF, Yang YJ, Xiao W, Fan J, Liu J *et al.* Interactions between N-ethylmaleimide-sensitive factor and GluR2 in the nucleus accumbens contribute to the expression of locomotor sensitization to cocaine. *J Neurosci* 2014; **34**: 3493–3508.
41 Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ *et al.* Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 2007; **131**: 391–404.
42 Feng Z, Liu L, Zhang C, Zheng T, Wang J, Lin M *et al.* Chronic restraint stress attenuates p53 function and promotes tumorigenesis. *Proc Natl Acad Sci USA* 2012; **109**: 7013–7018.
43 Lee PH, Perlis RH, Jung JY, Byrne EM, Rueckert E, Siburian R *et al.* Multi-locus genome-wide association analysis supports the role of glutamatergic synaptic transmission in the etiology of major depressive disorder. *Transl Psychiatry* 2012; **2**: e184.
44 Vollmann-Honsdorf GK, Flugge G, Fuchs E. Chronic psychosocial stress does not affect the number of pyramidal neurons in tree shrew hippocampus. *Neurosci Lett* 1997; **233**: 121–124.
45 Tong L, Prieto GA, Kramar EA, Smith ED, Cribbs DH, Lynch G *et al.* Brain-derived neurotrophic factor-dependent synaptic plasticity is suppressed by interleukin-1beta via p38 mitogen-activated protein kinase. *J Neurosci* 2012; **32**: 17714–17724.
46 Jiang B, Wang F, Yang S, Fang P, Deng ZF, Xiao JL *et al.* SKF83959 produces antidepressant effects in a chronic social defeat stress model of depression through BDNF-TrkB pathway. *Int J Neuropsychopharmacol* 2014; **18**: pii: pyu096.
47 Anacker C, Zunszain PA, Carvalho LA, Pariante CM. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology* 2011; **36**: 415–425.
48 Koo JW, Duman RS. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci USA* 2008; **105**: 751–756.
49 Yao JH, Ye SM, Burgess W, Zachary JF, Kelley KW, Johnson RW. Mice deficient in interleukin-1beta converting enzyme resist anorexia induced by central lipopolysaccharide. *Am J Physiol* 1999; **277**: R1435–R1443.
50 Bilbo SD, Biedenkapp JC, Der-Avakian A, Watkins LR, Rudy JW, Maier SF. Neonatal infection-induced memory impairment after lipopolysaccharide in adulthood is prevented via caspase-1 inhibition. *J Neurosci* 2005; **25**: 8000–8009.
51 Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 2002; **10**: 417–426.
52 Vigano E, Diamond CE, Spreafico R, Balachander A, Sobota RM, Mortellaro A. Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes. *Nat Commun* 2015; **6**: 8761.
53 Hu W, Zhang Y, Wu W, Yin Y, Huang D, Wang Y *et al.* Chronic glucocorticoids exposure enhances neurodegeneration in the frontal cortex and hippocampus via NLRP-1 inflammasome activation in male mice. *Brain Behav Immun* 2016; **52**: 58–70.
54 Alcocer-Gomez E, Ulecia-Moron C, Marin-Aguilar F, Rybkina T, Casas-Barquero N, Ruiz-Cabello J *et al.* Stress-induced depressive behaviors require a functional NLRP3 inflammasome. *Mol Neurobiol* 2016; **53**: 4874–4882.
55 Iwata M, Ota KT, Li XY, Sakaue F, Li N, Dutheil S *et al.* Psychological stress activates the inflammasome via release of adenosine triphosphate and stimulation of the purinergic type 2X7 receptor. *Biol Psychiatry* 2016; **80**: 12–22.
56 Momeni M, Ghorban K, Dadmanesh M, Khodadadi H, Bidaki R, Kazemi Arababadi M *et al.* ASC provides a potential link between depression and inflammatory disorders: a clinical study of depressed Iranian medical students. *Nord J Psychiatry* 2016; **70**: 280–284.
57 Koo JW, Duman RS. Interleukin-1 receptor null mutant mice show decreased anxiety-like behavior and enhanced fear memory. *Neurosci Lett* 2009; **456**: 39–43.
58 Tordera RM, Totterdell S, Wojcik SM, Brose N, Elizalde N, Lasheras B *et al.* Enhanced anxiety, depressive-like behaviour and impaired recognition memory in mice with reduced expression of the vesicular glutamate transporter 1 (VGLUT1). *Eur J Neurosci* 2007; **25**: 281–290.
59 Garcia-Garcia AL, Elizalde N, Matrov D, Harro J, Wojcik SM, Venzala E *et al.* Increased vulnerability to depressive-like behavior of mice with decreased expression of VGLUT1. *Biol Psychiatry* 2009; **66**: 275–282.
60 Inta D, Vogt MA, Perreau-Lenz S, Schneider M, Pfeiffer N, Wojcik SM *et al.* Sensorimotor gating, working and social memory deficits in mice with reduced expression of the vesicular glutamate transporter VGLUT1. *Behav Brain Res* 2012; **228**: 328–332.
61 Tzanoulinou S, Riccio O, de Boer MW, Sandi C. Peripubertal stress-induced behavioral changes are associated with altered expression of genes involved in excitation and inhibition in the amygdala. *Transl Psychiatry* 2014; **4**: e410.

62 Zink M, Vollmayr B, Gebicke-Haerter PJ, Henn FA. Reduced expression of glutamate transporters vGluT1, EAAT2 and EAAT4 in learned helpless rats, an animal model of depression. *Neuropharmacology* 2010; **58**: 465–473.
63 Huang GB, Zhao T, Muna SS, Bagalkot TR, Jin HM, Chae HJ *et al*. Effects of chronic social defeat stress on behaviour, endoplasmic reticulum proteins and choline acetyltransferase in adolescent mice. *Int J Neuropsychopharmacol* 2013; **16**: 1635–1647.
64 Matsuo N, Reijmers L, Mayford M. Spine-type-specific recruitment of newly synthesized AMPA receptors with learning. *Science* 2008; **319**: 1104–1107.
65 Hu W, Zhang M, Czeh B, Flugge G, Zhang W. Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. *Neuropsychopharmacology* 2010; **35**: 1693–1707.
66 Filipovic D, Zlatkovic J, Gass P, Inta D. The differential effects of acute vs. chronic stress and their combination on hippocampal parvalbumin and inducible heat shock protein 70 expression. *Neuroscience* 2013; **236**: 47–54.
67 Gemma C, Bachstetter AD, Cole MJ, Fister M, Hudson C, Bickford PC. Blockade of caspase-1 increases neurogenesis in the aged hippocampus. *Eur J Neurosci* 2007; **26**: 2795–2803.
68 Vereker E, Campbell V, Roche E, McEntee E, Lynch MA. Lipopolysaccharide inhibits long term potentiation in the rat dentate gyrus by activating caspase-1. *J Biol Chem* 2000; **275**: 26252–26258.
69 Curran BP, Murray HJ, O'Connor JJ. A role for c-Jun N-terminal kinase in the inhibition of long-term potentiation by interleukin-1beta and long-term depression in the rat dentate gyrus *in vitro*. *Neuroscience* 2003; **118**: 347–357.
70 Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T *et al*. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci* 2003; **23**: 8692–8700.
71 Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A *et al*. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* 2013; **493**: 674–678.
72 Distrutti E, O'Reilly JA, McDonald C, Cipriani S, Renga B, Lynch MA *et al*. Modulation of intestinal microbiota by the probiotic VSL#3 resets brain gene expression and ameliorates the age-related deficit in LTP. *PLoS One* 2014; **9**: e106503.
73 Gareau MG, Wine E, Rodrigues DM, Cho JH, Whary MT, Philpott DJ *et al*. Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* 2011; **60**: 307–317.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)