

EphB2 in the Medial Prefrontal Cortex Regulates Vulnerability to Stress

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The ephrin B2 (EphB2) receptor is a tyrosine kinase receptor that is associated with synaptic development and maturation. It has recently been implicated in cognitive deficits and anxiety. However, still unknown is the involvement of EphB2 in the vulnerability to stress. In the present study, we observed decreases in EphB2 levels and their downstream molecules in the medial prefrontal cortex (mPFC) but not in the orbitofrontal cortex (OFC) in mice that were susceptible to chronic social defeat stress. The activation of EphB2 receptors with EphrinB1-Fc in the mPFC produced stress-resistant and antidepressant-like behavioral effects in susceptible mice that lasted for at least 10 days. EphB2 receptor knockdown by short-hairpin RNA in the mPFC increased the susceptibility to stress and induced depressive-like behaviors in a subthreshold chronic social defeat stress paradigm. These behavioral effects were associated with changes in the phosphorylation of cofilin and membrane α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) trafficking and the expression of some synaptic proteins in the mPFC. We also found that EphB2 regulated stress-induced spine remodeling in the mPFC. Altogether, these results indicate that EphB2 is a critical regulator of stress vulnerability and might be a potential target for the treatment of depression.

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INTRODUCTION

Major depressive disorder (MDD) is a widespread psychiatric disorder that greatly influences public health (Krishnan and Nestler, 2008). Both genetic and environmental factors, such as stress, are involved in the etiology of depression (Kendler *et al.*, 1999). However, only a small percentage of individuals, both humans and rodents, develop mental illnesses (eg, posttraumatic stress disorder and depression) when exposed to extraordinary levels of stress and trauma (Golden *et al.*, 2011; Krishnan *et al.*, 2007; Yu *et al.*, 2012). Individuals who have the ability to perceive adversity as minimally threatening and consequently develop adaptive physiological and psychological responses to avoid negative social, psychological, and biological consequences of stressful events are identified as resilient (Russo *et al.*, 2012). A better understanding of the neural and molecular mechanisms that

mediate resistance to stress is important for identifying new therapeutic targets for depression.

Alterations in the medial prefrontal cortex (mPFC), a region that is sensitive to the detrimental effects of stress exposure, are implicated in the pathophysiology of depression (Arnsten, 2009). Chronic stress dampens mPFC glutamate transmission (Yuen *et al.*, 2012) and causes the dendritic atrophy of PFC neurons (Bloss *et al.*, 2011; Duman and Duman, 2015), consistent with decreases in PFC volume that have been reported in imaging studies of MDD patients (Drevets *et al.*, 1997; Rajkowska *et al.*, 1999). Moreover, the optogenetic activation of mPFC produces an antidepressant-like effect in mice that are subjected to chronic social defeat (Covington *et al.*, 2010; Vialou *et al.*, 2014), indicating that the mPFC may have an important role in regulating stress vulnerability.

The ephrin family of receptors is the largest class of receptor tyrosine kinases and classified as EphA and EphB according to the type of ligands to which they bind (Himanen and Nikolov, 2003). Ephrins are membrane-attached ligands that bind to Eph receptors and activate their tyrosine kinase catalytic domain. Ephrins and their receptors have critical roles in axon guidance (Brambilla and Klein, 1995) and topographic mapping in the developing nervous system (Klein, 2004). Ephrins and their receptors are also

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highly expressed in the adult nervous system. In mature brain synapses, EphB2 is enriched in large dendritic shafts and dendritic spines in the frontal cortex and hippocampus (Bouvier *et al*, 2008) and participate in regulating the distribution of glutamate receptors (GluRs) and formation of excitatory synapses (Kayser *et al*, 2006). EphB2 down-regulation leads to deficits in both the formation and maintenance of mature dendritic spines (Ethell *et al*, 2001; Irie and Yamaguchi, 2002; Shi *et al*, 2009). EphB2 also regulates synapse plasticity and contributes to emotional behaviors and cognitive deficits (Attwood *et al*, 2011; Cisse *et al*, 2011). However, still unknown is whether EphB2 regulates the vulnerability to stress.

In the present study, we investigated the role of EphB2 in the mPFC in regulating structural plasticity and vulnerability to stress in a model of chronic social defeat stress. Uncovering the molecular mechanisms that underlie resistance to stress may have therapeutic benefits for depression.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (6–8 weeks old) and male CD1 retired breeder mice (8-months old) were obtained from the Peking University Experimental Animal Center. The mice were housed under constant temperature ($23 \pm 2^\circ\text{C}$) and humidity ($50 \pm 5\%$) and maintained on a 12 h/12 h light/dark cycle with free access to food and water. All of the procedures were performed with approval from the Biomedical Ethics Committee for Animal Use and Protection of Peking University and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All of the behavioral tests and drug administrations were performed during the animals' dark phase.

Drugs

EphrinB1/Fc chimera from mouse (purity >95%) was purchased from Sigma (St Louis, MO, USA). To prepare clustered EphrinB1/Fc, 20 μg recombinant mouse EphrinB1-Fc chimera, which connects mouse EphrinB1 to the Fc portion of the human IgG1 that is histidine-tagged at the carboxyl terminus, was incubated with 48 mg goat anti-human IgG (Fc) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 30 μl of phosphate-buffered saline (PBS) that contained 1% heat-inactivated bovine serum albumin at 4°C for at least 1 h. For intracerebral injections, 0.5 μl of the prepared drug that contained 0.1 μg clustered Ephrin B1-Fc or IgG (Fc) was delivered (Yang *et al*, 2014).

Design, Construction, and Validation of Adenovirus Vectors for Knockdown of EphB2

Short-hairpin RNAs (shRNAs) were designed specifically for EphB2 (EphB2-shRNA, GGACCTTGTTTATAACATCAT; scramble, GGAATCTCATTCGATGCATAC). The construction and use of the self-complementing adenovirus vectors were based on our previous studies with minor modifications (Jian *et al*, 2014).

Surgery and Intracranial Injections

Sodium pentobarbital (60 mg/kg, i.p.) was used to anesthetize the mice before they were bilaterally implanted with permanent guide cannulae (outer diameter, 0.41 mm; inner diameter, 0.25 mm; RWD Life Science, Shenzhen, China) in the mPFC (anterior/posterior, +1.75 mm; medial/lateral, ± 0.75 mm; dorsal/ventral, -2.65 mm at 15° angle) and orbitofrontal cortex (OFC; anterior/posterior, +2.50 mm; medial/lateral, ± 0.70 mm; dorsal/ventral, -1.00 mm) (Covington *et al*, 2010; Faccidomo *et al*, 2008). The drugs were intracranially microinjected using 10- μl Hamilton syringes (Hamilton, Reno, NV, USA) that were connected via polyethylene-50 tubing (outer diameter, 0.61 mm; inner diameter, 0.28 mm; RWD Life Science) to injectors (outer diameter, 0.21 mm; inner diameter, 0.11 mm; RWD Life Science). For both the pharmacological and viral infusions, a total volume of 0.5 μl was infused into each side over 5 min, and the injection syringe was left in place for an additional 5 min to allow for diffusion. At the end of the experiments, the mice were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused. Cannula placements were assessed using Nissl staining of 20- μm thick sections under light microscopy. Subjects with misplaced cannulae were excluded from the statistical analysis.

Chronic Social Defeat Stress Paradigm

The chronic social defeat stress procedure was based on a previous study (Covington *et al*, 2011; Golden *et al*, 2011). C57BL/6 mice were subjected to chronic social defeat stress for 10 consecutive days. Every day, each mouse in the stressed group was introduced to the home cage of an unfamiliar resident for 5 min and physically defeated. Resident mice were CD1 breeders that were selected based on their attack latencies, which were reliably <30 s in three consecutive screening tests. After 5 min of physical interaction, the residents and intruders remained in sensory contact in the resident home cage for 24 h but were separated by a perforated Plexiglas partition. Each mouse in the stressed group was exposed to a new resident home cage each day. Control animals were housed in pairs in one cage and also separated by a perforated Plexiglas partition. The subthreshold chronic social defeat stress procedure was identical to the normal chronic social defeat stress procedure, with the exception that the procedure lasted for 3 consecutive days. The mice were removed from the study and immediately killed if repeated defeats led to the development of open wounds that exceeded 1 cm or severe infection.

Social Interaction Test

The social interaction test was performed according to previously established protocols (Golden *et al*, 2011). C57BL/6 mice were placed in an open field (42×42 cm²) with a small empty Plexiglas cage that was placed on one wall. The amount of time that the animal spent in the area around this cage was recorded over 2.5 min. A novel CD1 mouse was then introduced into the Plexiglas cage, and the procedure was repeated. Animals that spent more time in the interaction zone when a novel target mouse was present

compared with when the novel target mouse was absent were considered resilient, whereas animals that spent less time in the interaction zone when the novel target mouse was present were considered susceptible. The interaction ratio was calculated as the following: (interaction time, target present)/(interaction time, target absent). An interaction ratio of 1 was set as the cutoff. Mice with scores <1 were considered 'Susceptible', and mice with scores ≥ 1 were considered 'Resilient'.

Sucrose Preference Test

The sucrose preference test was performed according to previously established protocols (Covington *et al*, 2009; Shi *et al*, 2012; Suo *et al*, 2013). Animals were given two bottles that contained 1% sucrose for 2 days of adaptation. After adaptation, the mice were deprived of water for 24 h and then subjected to the sucrose preference test. In the sucrose preference test, animals were housed in individual cages for 12 h and had free access to two bottles that contained 1% sucrose or water. We counterbalanced the bottles across the left and right sides of the cages throughout the experiment. The position of the two bottles was changed every 6 h during the test. At the end of the test, we measured sucrose and water consumption (in milliliters) and calculated sucrose preference (%) as the ratio of sucrose consumption to sucrose plus water consumption.

Novelty-Suppressed Feeding Test

The novelty-suppressed feeding test was performed according to previously established protocols (Shen *et al*, 2010; Shi *et al*, 2012; Suo *et al*, 2013). Mice were deprived of food for 18 h and then placed in the corner of a Plexiglas box ($42 \times 42 \times 42 \text{ cm}^3$), and a pellet of food was placed on a white paper square ($6 \times 6 \text{ cm}^2$) in the center of the cage. Each test lasted 5 min, and the latency to approach the food and begin eating was recorded (in seconds) as the main test parameter. The latency to feed was scored when the mouse sat on the paper square and bit the pellet using its forepaws. Subsequent home cage food consumption over 5 min was the quantitative control measure for appetite.

Open Field Test

Locomotor activity was measured using the open field test as previously described (Han *et al*, 2015; Xue *et al*, 2015). Briefly, the apparatus consisted of a $42 \times 42 \times 42 \text{ cm}^3$ square arena that was divided into 25 equal squares ($8.4 \times 8.4 \text{ cm}^2$) on the floor of the arena. The mice were placed in the center of the apparatus, and the number of crossings into adjacent squares of the apparatus were counted for 5 min.

Tissue Sample Preparation

The procedure was based on our previous studies (Chai *et al*, 2014; Luo *et al*, 2015; Xue *et al*, 2012). After the last behavioral test, the mice were decapitated. The brains were quickly frozen in -60°C N-hexane, and bilateral tissue punches of the mPFC and OFC (8-gauge) were obtained and placed in a solution that contained 0.32 M sucrose, 20 mM HEPES (pH 7.4), 1 mM ethylenediaminetetraacetic

acid (EDTA), $1 \times$ protease inhibitor cocktail, 5 mM NaF, and 1 mM sodium vanadate. After being homogenized by an electrical disperser (Wiggenhauser, Sdn Bnd, Los Lunas, NM, USA), the homogenate was centrifuged for 10 min at 2800 rotations per minute (r.p.m.) at 4°C . The pellet that contained nuclei and large cellular debris was discarded. The supernatant was centrifuged at 12 000 r.p.m. for 10 min. After centrifugation, the supernatant (cytosolic fraction) was removed, and the pellet (crude synaptosomal fraction) was resuspended and sonicated in protein lysis buffer (50 mM Tris-HCl (pH 7.50) 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 2 mM EDTA, 1 mM NaVO_3 , 5 mM NaF, and $1 \times$ protease inhibitor cocktail). The protein concentrations of all of the samples from the crude synaptosomal fraction were determined using the BCA assay kit (Beyotime Biotechnology, Shanghai, China).

Western Blotting

The assay procedures were based on our previous studies (Lu *et al*, 2005; Zhu *et al*, 2013). Samples from the crude synaptosomal fraction were subjected to SDS-polyacrylamide gel electrophoresis (12.5% acrylamide/0.27% *N,N'*-methylenebisacrylamide resolving gel) for approximately 30 min at 80 V in stacking gel and approximately 1 h at 120 V in resolving gel. Proteins were electrophoretically transferred to Immobilon-P transfer membranes (Millipore, Bedford, MA, USA) at 0.25 A for 3 h. The membranes were washed with TBST (Tris-buffered saline plus 0.05% Tween-20, pH 7.4) before dipping in blocking buffer (5% skimmed dry milk in TBST) overnight at 4°C . The membranes were then incubated for 1 h at room temperature with anti-EphB2 antibody (1:1000; Abcam, Cambridge, UK; catalog no. 5418), anti-phospho-cofilin antibody (1:1000; Abcam; catalog no. 12866), anti-cofilin antibody (1:1000; Abcam; catalog no. 42824), anti-GluR1 antibody (1:1000; Abcam; catalog no. ab109450), anti-GluR2 antibody (1:1000; Abcam; catalog no. ab52932), anti-PSD95 antibody (1:1000; Abcam; catalog no. ab18258), anti-Synapsin I antibody (1:1000; Abcam; catalog no. ab64581), or anti-GAPDH antibody (1:1000, Abcam; catalog no. 181602). After shaking in 4×6 min washes in TBST buffer, the blots were incubated for 45 min at room temperature with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit or mouse IgG; Santa Cruz Biotechnology and Vector Labs, respectively) diluted 1:3000 in blocking buffer. The blots were then shaken in 4×6 min washes in TBST. Afterward, the blots were incubated with a layer of Super Signal enhanced chemiluminescence substrate mixture (Pierce Biotechnology, Rockford, IL, USA) for 1 min at room temperature. The excess mixture was dripped off before the blots were wrapped with a clean piece of plastic wrap (no bubbles between blot and wrap), and the blots were then screened using the ChemiDoc MP System (Bio-Rad, Hercules, CA, USA) for 5–60 s. The band intensities were quantified with Quantity One 4.4.0 software from Bio-Rad (Hercules, CA, USA).

Golgi-Cox Staining

The Golgi-Cox impregnation procedure was based on a previous study (Han *et al*, 2015; Restivo *et al*, 2005). The mice were perfused with 0.9% saline, and the brains were

removed and placed in Golgi–Cox solution (1.04% potassium dichromate, 1.04% mercury chloride, and 0.83% potassium chromate, dissolved in double-distilled water) at room temperature for 14 days. The solution was refreshed every 3 days, immersed in a 30% sucrose solution for 5 days, and then coronally sectioned (100 μm) using a vibratome. Staining was developed by a 1-min rinse in water, 30 min in 16% ammonia, and a 1-min rinse in water followed by dehydration for 1 min in 50% ethanol, 1 min in 70% ethanol, 1 min in 90% ethanol, 1 min in 100% ethanol, and 15 min in CXA solution (trichloromethane:dimethylbenzene:ethanol, 1:1:1). The sections were then coated with transparent xylene and sealed with neutral balsam.

All of the images were captured with an Olympus BX53 microscope using a $\times 100$ oil-immersion objective. For the quantitative analysis of spine density, the spines were counted along dendritic segments that were chosen from secondary and tertiary dendrites. Dendrite length was measured using the NIH ImageJ software, and the number of dendritic spines was counted by two trained observers who were blind to the experimental conditions. The average number of spines per 10 μm of dendrite was calculated. For each group, we examined 6–10 neurons per mouse and 3–5 mice per group. Three segments for each neuron were selected for analysis.

Histology

After the behavioral experiments, the mice were anesthetized and perfused with 0.01 M PBS and 4% paraformaldehyde, pH 7.4. The brains were then extracted and postfixed in 4% paraformaldehyde for 24 h. Subsequently, the brains were cryoprotected in 30% sucrose in 0.2 M phosphate buffer and frozen.

Cannula placements were assessed using Nissl staining with a section thickness of 20 μm under light microscopy. The locations of the cannulae are shown in Supplementary Figure S8. Additionally, the brains were coronally sectioned at 20 μm using a sliding microtome, and the brain slices were counterstained with DAPI and examined using an Olympus BX53 fluorescent microscope to evaluate enhanced green fluorescent protein expression in the mPFC and OFC (Han *et al*, 2015; Xue *et al*, 2015). Mice with misplaced cannulae were excluded from the statistical analysis.

Statistical Analysis

All of the statistical analyses were performed using the SPSS 17.0 software (SPSS, Chicago, IL, USA). The data are expressed as mean \pm SEM and were analyzed using analysis of variance (ANOVA) with appropriate between- and within-subject factors for each experiment (see Results section). Significant main effects and interactions ($p < 0.05$) in the factorial ANOVAs were further analyzed using one-way ANOVAs and Least Significant Difference *post hoc* tests.

RESULTS

Chronic Social Defeat Stress Decreased EphB2 Signaling in the mPFC in Susceptible Mice

Chronic social defeat stress is an etiologically valid approach to model the symptomatology of stress-related disorders and

can distinguish between the susceptible and resilient individuals (Golden *et al*, 2011; Krishnan *et al*, 2007). We first examined the effects of chronic social defeat stress on EphB2 and its downstream signaling. Mice were randomly assigned to a non-stressed group and stressed group. The stressed group was subjected to 10 consecutive days of chronic social defeat stress, and mice in the non-stressed group were normally housed in their home cages for 10 days. The mice then underwent the social interaction test, and stressed mice were divided into resilient and susceptible subgroups according to their different behavioral performance. Subsequently, all three groups underwent the sucrose preference test and novelty-suppressed feeding test 1 and 2 days after the social interaction test (Figure 1a).

Stressed mice were divided into susceptible and resilient groups based on the measure of social avoidance, in which susceptible animals exhibited a significant reduction of social interaction compared with both the non-stressed and resilient groups (one-way ANOVA, $F_{2,40} = 15.655$, $p < 0.05$; Figure 1b, left column). Multivariate ANOVAs revealed that susceptible mice spent less time in the interaction zone (phenotype \times target interaction, $F_{2,76} = 16.825$, $p < 0.05$; Figure 1b, right column) and more time in the corner zone (phenotype \times target interaction, $F_{2,76} = 19.617$, $p < 0.05$; Supplementary Figure S1A) when the target was present compared with the non-stressed group. Compared with the non-stressed group, the susceptible group presented anhedonia in the sucrose preference test (one-way ANOVA, $F_{2,40} = 16.357$, $p < 0.05$; Figure 1c, left column) and anxiety-like behavior in the novelty-suppressed feeding test (one-way ANOVA, $F_{2,40} = 14.519$, $p < 0.05$; Figure 1d, left column). Chronic social defeat stress did not alter fluid consumption ($p > 0.05$; Figure 1c, right column) or total food intake ($p > 0.05$; Figure 1d, right column) in the three groups. These results indicate that susceptible mice exhibited a depression-like phenotype.

We found that chronic social defeat stress significantly decreased EphB2 levels in the mPFC in susceptible mice (one-way ANOVA, $F_{2,36} = 25.98$, $p < 0.05$; Figure 1e) but not in resilient mice. Previous studies showed that EphB2 regulates cofilin activity (Shi *et al*, 2009; Simon *et al*, 2009). Therefore, we next examined the phosphorylation of cofilin in the mPFC and OFC after chronic social defeat stress. The one-way ANOVA revealed that chronic social defeat stress significantly reduced the phosphorylation of cofilin ($F_{2,36} = 31.59$, $p < 0.05$; Figure 1e) in the mPFC in susceptible mice. A previous study showed that synaptic strength and α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor regulation mediate stress resilience (Vialou *et al*, 2010). Thus we examined the expression of postsynaptic density (PSD95), synapsin I, GluR1, and GluR2 after chronic social defeat stress. The one-way ANOVA revealed that chronic social defeat stress decreased the expression of PSD95 ($F_{2,36} = 9.42$, $p < 0.05$; Figure 1e), synapsin I ($F_{2,36} = 12.19$, $p < 0.05$; Figure 1e), GluR1 ($F_{2,36} = 30.15$, $p < 0.05$; Figure 1e), and GluR2 ($F_{2,36} = 58.60$, $p < 0.05$; Figure 1e) in the mPFC in susceptible mice but not in resilient mice (all $p > 0.05$; Figure 1e).

Additionally, chronic social defeat stress had no effect on the expression of EphB2, the phosphorylation of cofilin, cofilin, PSD95, synapsin I, or membranous GluR1 or GluR2 in the OFC (all $p > 0.05$; Figure 1f). These results indicate

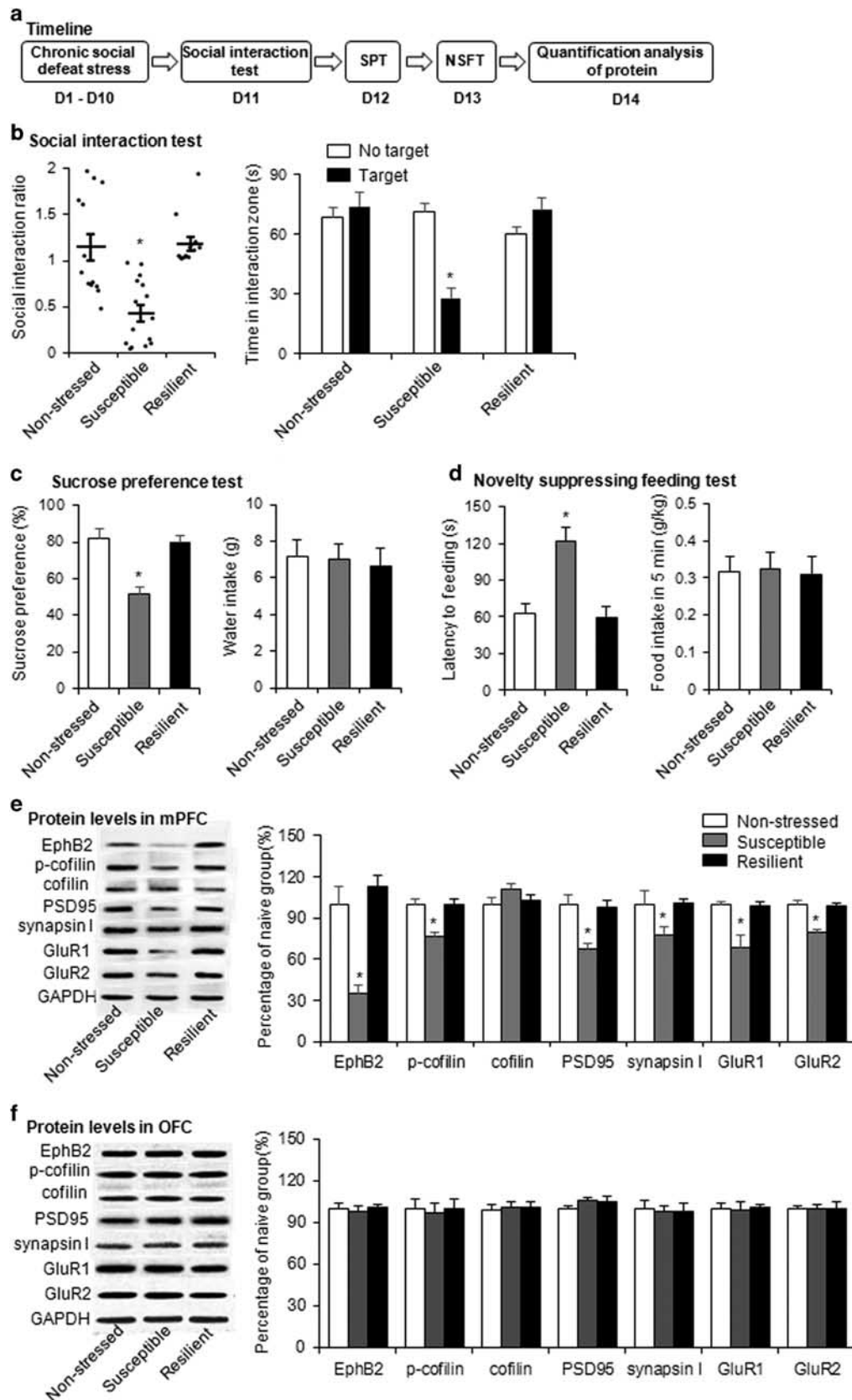


Figure 1 Chronic social defeat stress decreased EphB2 signaling in the mPFC in susceptible mice. (a) Experimental timeline of chronic social defeat stress. (b) Social interaction ratio and time spent in the interaction zone in the social interaction test. (c) Sucrose preference and water intake in the sucrose preference test (SPT). (d) Latency to feed and food intake over 5 min in the novelty-suppressed feeding test (NSFT) after 10 days of chronic social defeat stress ($n = 13-15$ per group). (e, f) EphB2 and downstream protein levels and representative western blottings in the mPFC and OFC after chronic social defeat stress ($n = 10-14$ per group). The downstream proteins included phosphorylated cofilin, cofilin, PSD95, synapsin I, and membranous GluR1 and GluR2. * $p < 0.05$, compared with the non-stressed group.

that the susceptibility to stress is associated with a decrease in EphB2 signaling in the mPFC but not in the OFC.

Activation of EphB2 in the mPFC Reversed the Susceptibility to Stress

We next examined the effects of EphB2 activation on depressive-like behaviors after chronic social defeat stress. EphrinB1/Fc and PBS/Fc were constructed and microinfused in the mPFC or OFC in both susceptible and non-stressed mice. The mice were then subjected to the social interaction test, sucrose preference test, and novelty-suppressed feeding test 1 h after the infusions (Figure 2a) and 10 days after the infusions (Figure 3a). One hour after the intra-mPFC microinjection of EphrinB1/Fc, the social interaction ratio was significantly increased in the susceptible group (two-way ANOVA, main effect of stress, $F_{1,39} = 13.683$, $p < 0.05$; main effect of drug, $F_{1,39} = 24.007$, $p < 0.05$; stress \times drug interaction, $F_{1,39} = 8.404$, $p < 0.05$; Figure 2b, left column), and a similar effect was found 10 days after the infusions of EphrinB1/Fc (two-way ANOVA, main effect of stress, $F_{1,39} = 10.228$, $p < 0.05$; main effect of drug, $F_{1,39} = 5.629$, $p < 0.05$; stress \times drug interaction, $F_{1,39} = 5.394$, $p < 0.05$; Figure 3b, left column). Multivariate ANOVAs revealed that the susceptible mice that received EphrinB1/Fc microinjections spent more time in the interaction zone (1 h after infusion: stress \times drug \times target interaction, $F_{1,78} = 13.999$, $p < 0.05$, Figure 2b, right column; 10 days after infusion: stress \times drug \times target interaction, $F_{1,78} = 9.556$, $p < 0.05$, Figure 3b, right column) and spent less time in the corner zone (1 h after infusion: stress \times drug \times target interaction, $F_{1,78} = 6.903$, $p < 0.05$, Supplementary Figure S2B; 10 days after infusion: stress \times drug \times target interaction, $F_{1,78} = 10.927$, $p < 0.05$, Supplementary Figure S3B) when the target was present compared with susceptible mice that received PBS/Fc microinjections. The microinjection of EphrinB1/Fc in the mPFC also produced antidepressant- and anxiolytic-like effects in the sucrose preference test and novelty-suppressed feeding test 1 h after infusion (Figure 2c and d) and 10 days after infusion (Figure 3c and d). The EphrinB1/Fc microinjected mice exhibited higher sucrose preference (1 h after infusion: two-way ANOVA, stress \times drug interaction, $F_{1,39} = 13.699$, $p < 0.05$, Figure 2c; 10 days after infusion: two-way ANOVA, stress \times drug interaction, $F_{1,39} = 9.429$, $p < 0.05$, Figure 3c). The microinjection of EphrinB1/Fc also reversed the stress-induced increase in the latency to feed (two-way ANOVA, stress \times drug interaction, $F_{1,39} = 8.165$, $p < 0.05$; Figure 2d), and similar effect was found 10 days after infusion (two-way ANOVA, stress \times drug interaction, $F_{1,39} = 6.622$, $p < 0.05$; Figure 3d). Locomotor activity was unaffected by the EphrinB1/Fc infusions (Figures 2e and 3e). The microinfusion of EphrinB1/Fc in the OFC in susceptible mice had no effect on social avoidance, depressive-, or anxiety-like behavior in the social interaction test, sucrose preference, or novelty-suppressed feeding (all $p > 0.05$; Figure 4 and Supplementary Figure S4). These results indicate that EphB2 in the mPFC but not in the OFC mediates stress-resistant, antidepressant, and anxiolytic responses.

We next investigated the potential mechanisms that underlie the pro-resilient effect of EphB2 activation. We measured the expression of downstream proteins of EphB2

in the mPFC after the EphrinB1/Fc microinfusion (Figure 2a). The activation of EphB2 in the mPFC reversed the decrease in p-cofilin (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 48.53$, $p < 0.05$; Figure 2f) that was induced by chronic social defeat stress in the susceptible group but not in the non-stressed group. The decreases in synaptic proteins that are associated with synaptic strength, including PSD95 (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 9.29$, $p < 0.05$; Figure 2f), synapsin I (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 16.902$, $p < 0.05$; Figure 2f), GluR1 (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 28.83$, $p < 0.05$; Figure 2f), and GluR2 (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 28.46$, $p < 0.05$; Figure 2f), were also rescued by EphB2 activation.

The increases in the expression of p-cofilin (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 33.65$, $p < 0.05$), PSD95 (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 12.87$, $p < 0.05$), synapsin I (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 13.29$, $p < 0.05$), membrane GluR1 (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 18.05$, $p < 0.05$), and membrane GluR2 (two-way ANOVA, stress \times drug interaction, $F_{1,36} = 14.88$, $p < 0.05$) were also found 10 days after EphrinB1/Fc application, which may explain the prolonged behavioral effects of EphB2 activation in the mPFC (Figure 3f).

Reduction of EphB2 in the mPFC Promoted Stress Susceptibility

To further investigate the impact of EphB2 knockdown on stress vulnerability, we utilized a subthreshold chronic social defeat stress paradigm that did not induce depressive-like behaviors. We first examined the efficiency of EphB2 knockdown by adenovirus-mediated shRNA (Figure 5b). One week after the infusion of AdV_{shEphB2} or AdV_{Scramble} in the mPFC, the mice were decapitated for the western blotting assays (Figure 5a). We found that EphB2 expression levels in the mPFC were significantly decreased in the AdV_{shRNA} group compared with the AdV_{Scramble} group (one-way ANOVA, $F_{1,15} = 40.63$, $p < 0.05$; Figure 5c). A similar decrease in EphB2 expression was detected in the OFC after local AdV-shEphB2 infusion (one-way ANOVA, $F_{1,15} = 50.12$, $p < 0.05$; Figure 6a and b).

Mice that received AdV_{shRNA} or AdV_{Scramble} infusion in the mPFC were subjected to subthreshold chronic social defeat stress and underwent behavioral tests on the following 3 days (Figure 5d). The mice that received an intra-PFC infusion of AdV_{shRNA} exhibited significant social avoidance in the social interaction test (two-way ANOVA, stress \times vector interaction, $F_{1,39} = 4.280$, $p < 0.05$; Figure 5e), anhedonia in the sucrose preference test (two-way ANOVA, stress \times vector interaction, $F_{1,39} = 4.795$, $p < 0.05$; Figure 5f), and anxiety-like behavior in the novelty-suppressed feeding test (two-way ANOVA, stress \times vector interaction, $F_{1,39} = 6.622$, $p < 0.05$; Figure 5g) after subthreshold social defeat stress. Multivariate ANOVAs revealed that subthreshold-stressed mice that received AdV_{shRNA} microinjections spent less time in the interaction zone (stress \times vector \times target interaction, $F_{1,78} = 2.941$, $p = 0.09$; Supplementary Figure S5A) and spent more time in the corner zone (stress \times vector \times target interaction, $F_{1,78} = 6.654$, $p < 0.05$; Supplementary Figure S5B) when the target was present

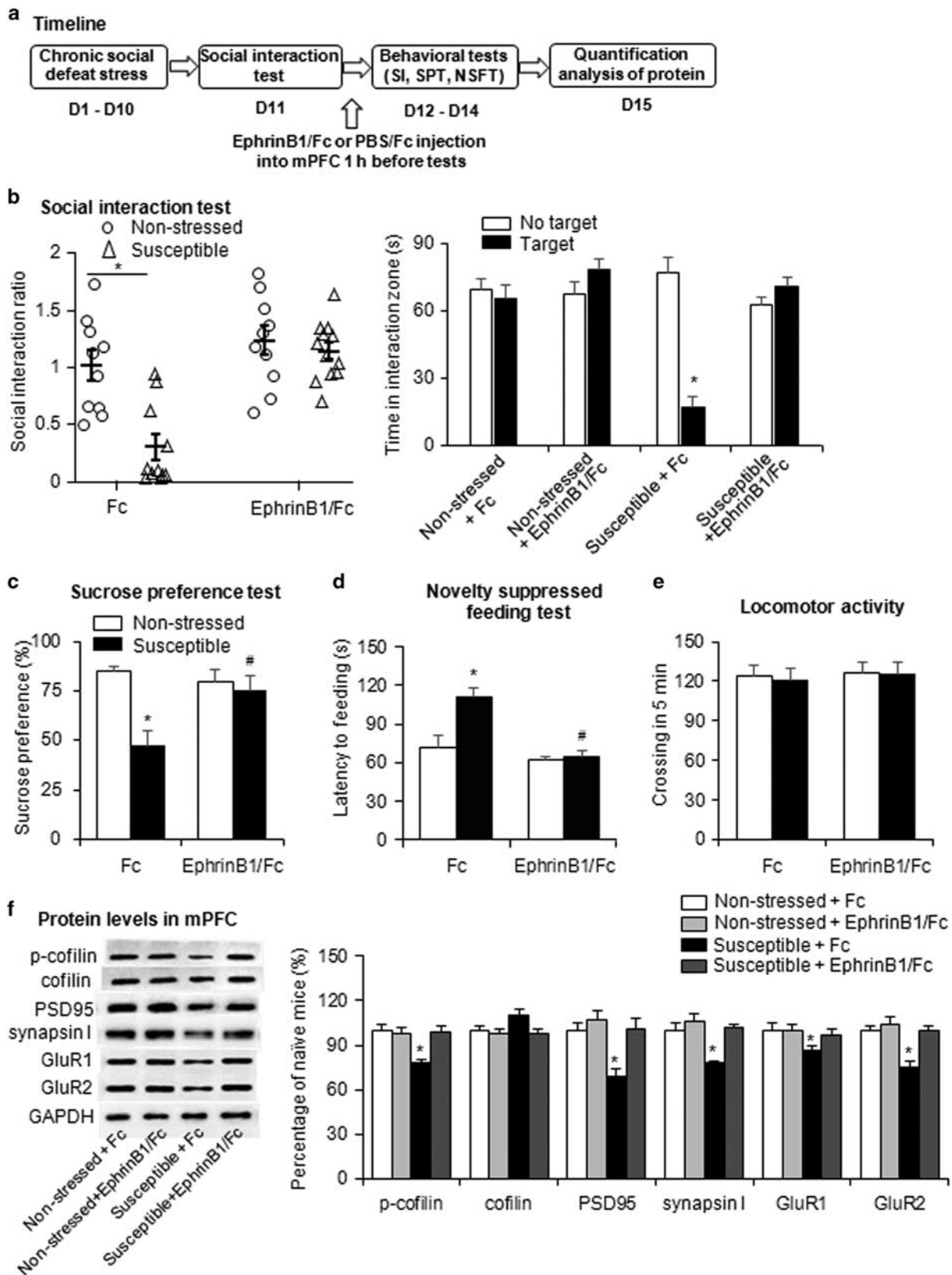
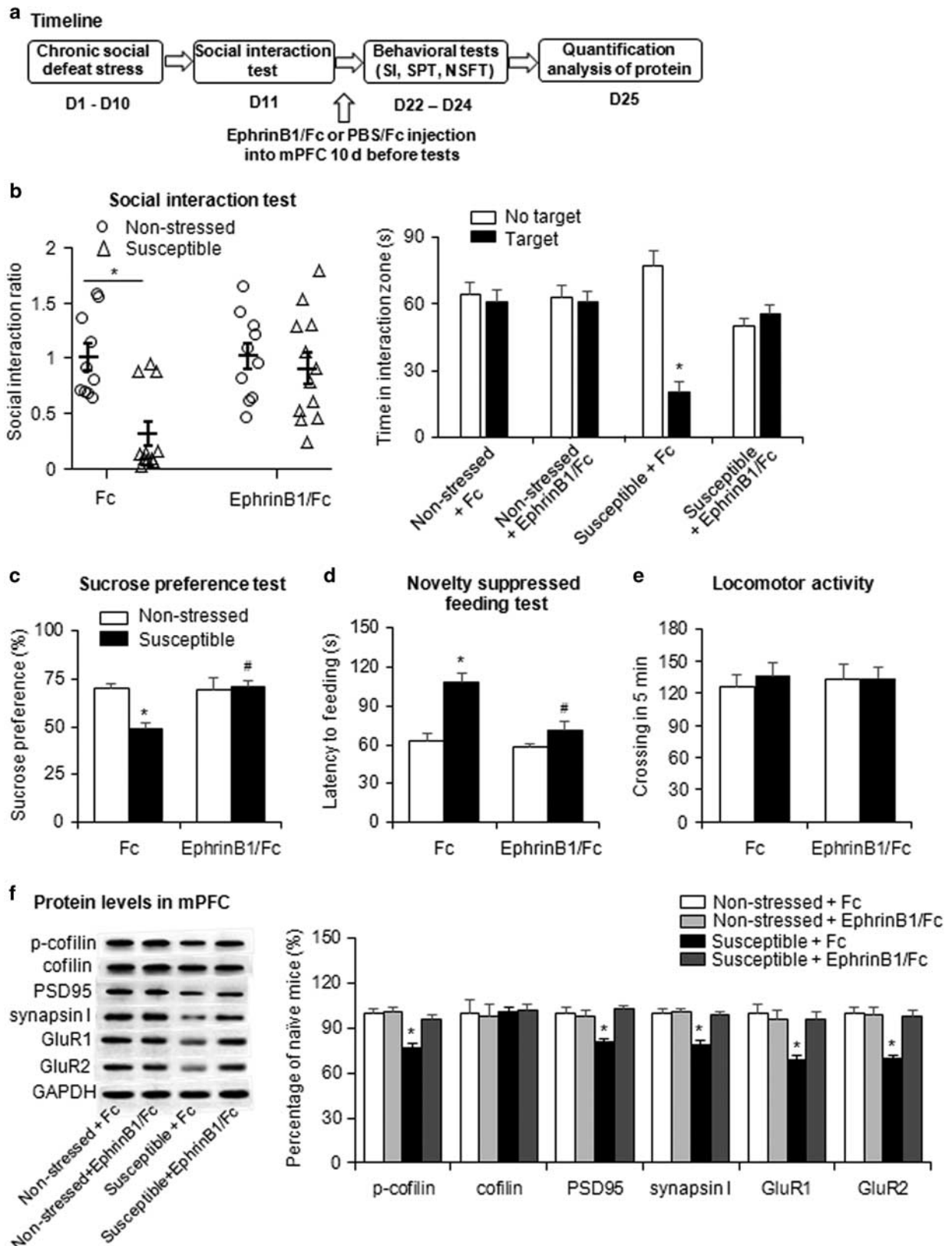


Figure 2 Activation of EphB2 in the mPFC improved depressive-like behaviors in susceptible mice. (a) Experimental timeline of chronic social defeat stress and EphrinB1/Fc microinjection in the mPFC 1 h before the behavioral tests. (b) Social interaction ratio and time spent in the interaction zone in the social interaction (SI) test. (c) Sucrose preference in the sucrose preference test (SPT). (d) Latency to feed over 5 min in the novelty-suppressed feeding test (NSFT). (e) Number of crossings during the 5 min open field test 1 h after EphrinB1/Fc microinjection in the mPFC ($n = 10-12$ per group). (f) EphB2 downstream proteins levels in the mPFC and representative western blottings 1 h after the microinjection ($n = 9-11$ per group). The EphB2 downstream proteins included phosphorylated cofilin, cofilin, PSD95, synapsin I, and membranous GluR1 and GluR2. * $p < 0.05$, compared with the non-stressed Fc group; # $p < 0.05$, compared with the susceptible Fc group.

compared with subthreshold-stressed mice that received AdV_{Scramble} microinjections. Mice that received AdV_{Scramble} did not exhibit these depressive-like behaviors after subthreshold social defeat stress (all $p > 0.05$; Figure 5e-g).

Locomotor activity was unaffected by EphB2 knockdown via adenovirus-mediated shRNA (Supplementary Figure S5D). EphB2 knockdown in the OFC had no significant effects on social interaction, sucrose preference, or the latency to feed



after subthreshold social defeat stress (all $p > 0.05$; Figure 6c–e and Supplementary Figure S6A and B).

We next examined the molecular mechanisms that are responsible for the increase in the susceptibility to stress

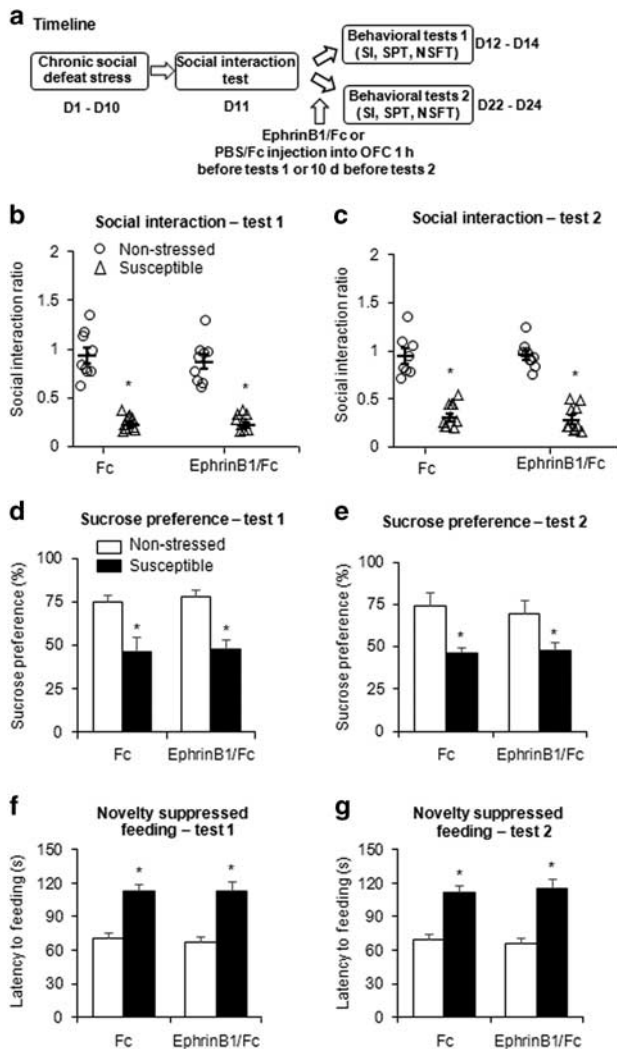


Figure 4 Activation of EphB2 in the OFC did not change depressive-like behaviors in susceptible mice. (a) Experimental timeline of EphrinB1/Fc microinjection and chronic social defeat stress. (b) Social interaction ratio in the social interaction (SI) test. (d) Sucrose preference in the sucrose preference test (SPT). (f) Latency to feed over 5 min in the novelty-suppressed feeding test (NSFT) 1 h after EphrinB1/Fc microinjection in the OFC (tests 1). (c) Social interaction ratio in the SI test. (e) Sucrose preference in the SPT. (g) Latency to feed over 5 min in the NSFT 10 days after EphrinB1/Fc microinjection in the OFC (tests 2). * $p < 0.05$, compared with the non-stressed Fc group. $n = 8–10$ per group.

after EphB2 knockdown. We found that AdV_{shRNA} microinjection decreased the EphB2 expression levels in both the non-stressed groups and subthreshold stress groups (two-way ANOVA, stress \times vector interaction, $F_{1,37} = 46.79$, $p < 0.05$; Figure 5h). EphB2 knockdown by AdV_{shRNA} microinfusion also decreased the expression of p-cofilin (two-way ANOVA, stress \times vector interaction, $F_{1,37} = 42.44$, $p < 0.05$; Figure 5h), PSD95 (two-way ANOVA, stress \times vector interaction, $F_{1,37} = 20.51$, $p < 0.05$; Figure 5h), synapsin I (two-way ANOVA, stress \times vector interaction, $F_{1,37} = 10.42$, $p < 0.05$; Figure 5h), GluR1 (two-way ANOVA, stress \times vector interaction, $F_{1,37} = 21.96$, $p < 0.05$; Figure 5h), and GluR2 (two-way ANOVA, stress \times vector interaction, $F_{1,37} = 28.12$, $p < 0.05$; Figure 5h) in the subthreshold stress groups but not in the non-stressed groups.

Overall, these results indicate that EphB2 knockdown in the mPFC but not in the OFC increased the susceptibility to stress, which was associated with decreases in the phosphorylation levels of cofilin and the expression of some synaptic proteins.

EphB2 Regulates Stress-Induced Dendritic Spine Remodeling in the mPFC

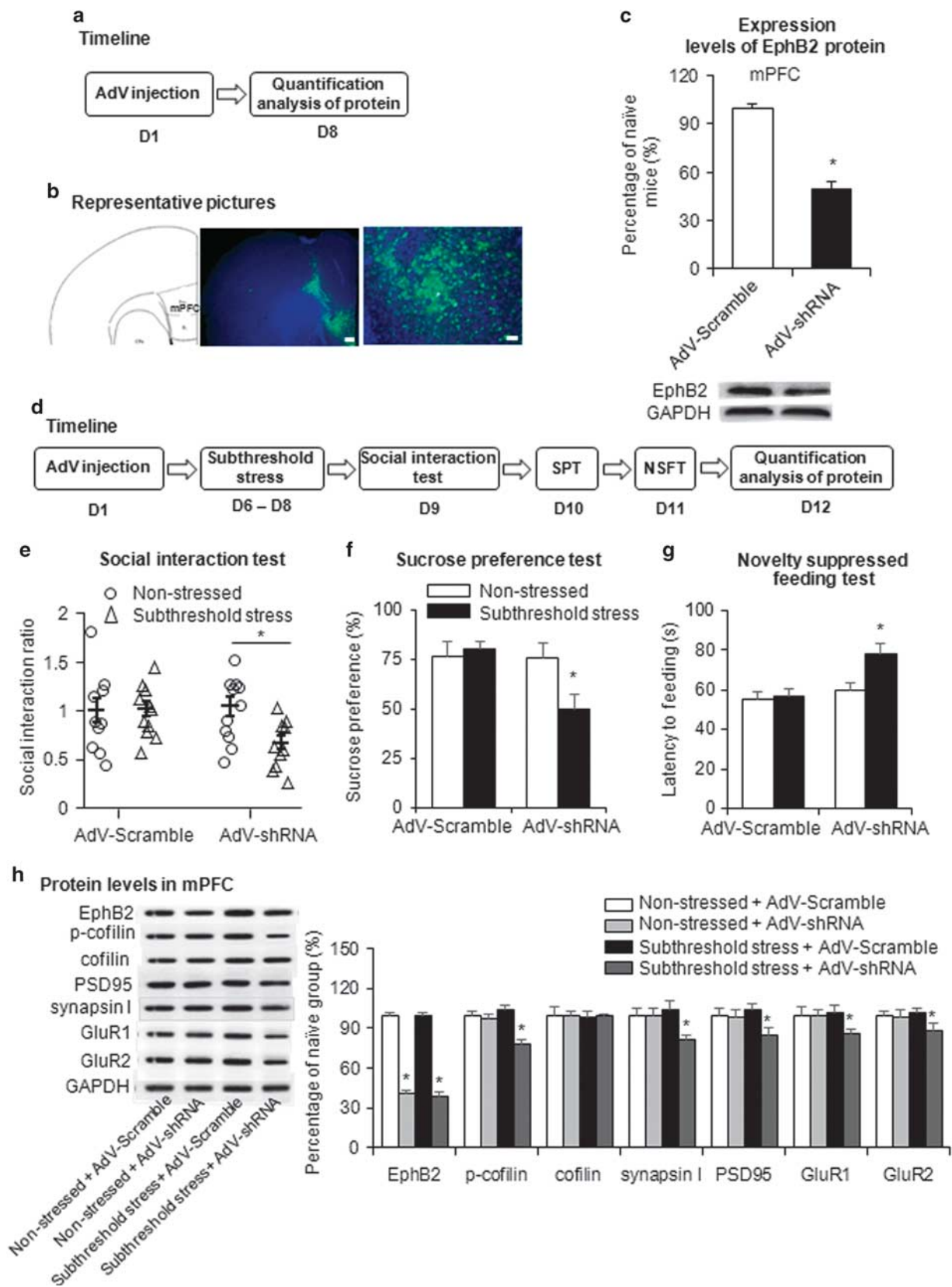
Previous studies showed that chronic stress decreased the density and function of spine synapses in the mPFC (Li *et al*, 2010; Radley *et al*, 2005; Radley *et al*, 2006). EphB2 has been reported to control the stability of mature dendritic spines partially by suppressing cofilin activity (Shi *et al*, 2009). We investigated the effects of modulating EphB2 signaling in the mPFC on the stress-induced decrease in spine density. Two groups of mice (susceptible group and non-stressed group) were microinjected with EphrinB1/Fc or Fc in the mPFC. One hour later, the mice were perfused, and brain tissues were collected for Golgi-Cox staining (Figure 7a). We found that chronic social defeat stress significantly decreased total spine density in the mPFC in susceptible mice, and EphrinB1/Fc microinfusion in the mPFC in susceptible mice reversed the decrease in total spine density (two-way ANOVA, stress \times drug interaction, $F_{1,116} = 4.018$, $p < 0.05$; Figure 7b and Supplementary Figure S7B).

Next we assessed the effects of EphB2 knockdown on the spine density of mPFC neurons using a subthreshold chronic social defeat stress model. Mice received a microinjection of AdV_{shEphB2} or AdV_{Scramble} in the mPFC and were subjected to 3 days of subthreshold stress. One hour later, they were killed, and brain tissues were collected for Golgi-Cox staining (Figure 7c). EphB2 knockdown in the mPFC decreased total spine density (two-way ANOVA, stress \times vector interaction, $F_{1,116} = 3.953$, $p < 0.05$; Figure 7d and Supplementary Figure S7D) after subthreshold social defeat

Figure 3 Activation of EphB2 in the mPFC produced long-term antidepressant-like effects. (a) Experimental timeline of chronic social defeat stress and EphrinB1/Fc microinjection in the mPFC 10 days before the behavioral tests. (b) Social interaction ratio and time spent in the interaction zone in the social interaction (SI) test. (c) Sucrose preference in the sucrose preference test (SPT). (d) Latency to feed over 5 min in the novelty-suppressed feeding test (NSFT). (e) Number of crossings during the 5 min open field test 10 days after EphrinB1/Fc microinjection in the mPFC ($n = 10–12$ per group). (f) EphB2 downstream proteins levels in the mPFC and representative western blottings 10 days after the microinjection ($n = 9–11$ per group). The EphB2 downstream proteins included phosphorylated cofilin, cofilin, PSD95, synapsin I, and membranous GluR1 and GluR2. * $p < 0.05$, compared with the non-stressed Fc group; # $p < 0.05$, compared with the susceptible Fc group.

stress. Neither subthreshold social defeat stress nor EphB2 knockdown in non-stressed mice affected total spine density (Figure 7d). Altogether, these results indicate that the

decrease in spine density in the mPFC was associated with susceptibility to stress, and EphB2 downregulation in the mPFC increased the vulnerability to stress.



DISCUSSION

In the present study, we found that chronic social defeat stress decreased the expression of EphB2 and downstream proteins in the mPFC in susceptible mice but not in resilient mice. EphB2 activation in the mPFC promoted resilience to stress, and this effect persisted for at least 10 days, whereas EphB2 knockdown induced depressive-like behaviors in the subthreshold social defeat stress paradigm. These behavioral effects were associated with changes in the phosphorylation of cofilin and the expression of some synaptic proteins in the mPFC. Moreover, we found that EphB2 activation reversed

the decrease in spine density in the mPFC in susceptible mice that was induced by chronic social defeat stress. EphB2 knockdown decreased the spine density of mPFC neurons in the subthreshold social defeat stress paradigm, which is not normally sufficient to decrease spine density. Altogether, our results indicate that EphB2 signaling in the mPFC is critical for regulating structural plasticity and the vulnerability to stress, and we propose a model that depicts the possible signaling cascade of EphB2 that regulates stress vulnerability (Figure 8).

The mPFC is sensitive to the detrimental effects of stress, and its dysfunction has been implicated in the pathophysiology

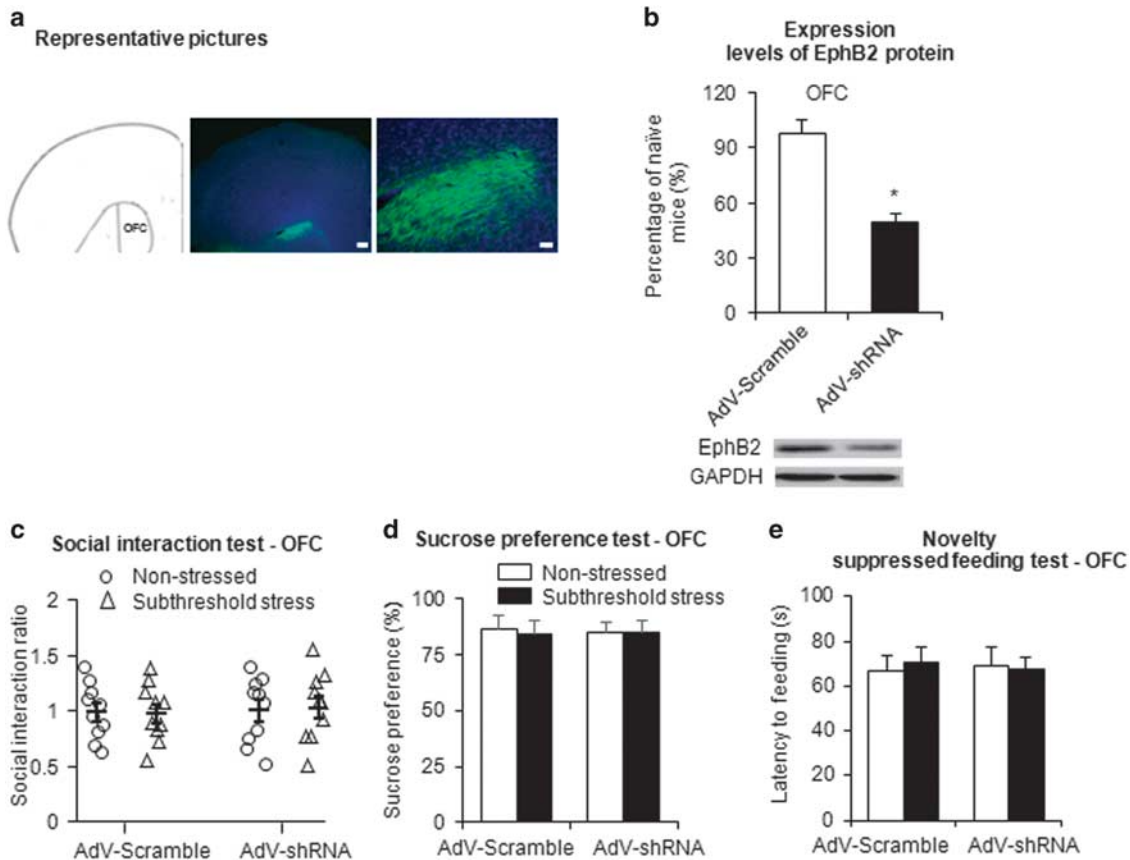


Figure 6 Knockdown of EphB2 in the OFC had no impact on the behavioral effects of subthreshold chronic social defeat stress. (a) Representative photographs of the injection sites and coronal brain sections in the OFC. The figure shows representative micrographs of adenovirus vector-mediated enhanced green fluorescent protein (eGFP; green), counterstained with DAPI, after OFC microinjection. Scale bars = 200 μ m (low-power images) and 50 μ m (high-power images). (b) EphB2 expression in the OFC in mice that were microinfused with AdV_{Scramble} or AdV_{shRNA}, quantified by western blotting. * $p < 0.05$, compared with the AdV_{Scramble} group. $n = 8$ per group. (c) Social interaction ratio in the social interaction test. (d) Sucrose preference in the sucrose preference test. (e) Latency to feed over 5 min in the novelty-suppressed feeding test after intra-OFC microinjection of AdV_{Scramble} or AdV_{shRNA} ($n = 9-10$ per group).

Figure 5 Knockdown of EphB2 in the mPFC induced depressive-like behaviors in the subthreshold chronic social defeat stress paradigm. (a) Experimental timeline for EphB2 detection. (b) Representative photographs of the injection sites and coronal brain sections in the mPFC. The figure shows representative micrographs of adenovirus vector-mediated enhanced green fluorescent protein (eGFP; green), counterstained with DAPI, after mPFC microinjection. Scale bars = 200 μ m (low-power images) and 50 μ m (high-power images). (c) EphB2 expression in the mPFC in mice that were microinfused with AdV_{Scramble} or AdV_{shRNA}, quantified by western blotting. * $p < 0.05$, compared with the AdV_{Scramble} group. $n = 8$ per group. (d) Experimental timeline for adenovirus microinjection and subthreshold chronic social defeat stress. (e) Social interaction ratio in the social interaction test. (f) Sucrose preference in the sucrose preference test (SPT). (g) Latency to feed over 5 min in the novelty-suppressed feeding test (NSFT) after intra-mPFC microinjection of AdV_{Scramble} or AdV_{shRNA} ($n = 10-11$ per group). (h) EphB2 and downstream protein levels in the mPFC and representative western blottings ($n = 10-11$ per group). The EphB2 downstream proteins included phosphorylated cofilin, cofilin, PSD95, synapsin I, and membranous GluR1 and GluR2. * $p < 0.05$, compared with subthreshold stress AdV_{Scramble} group.

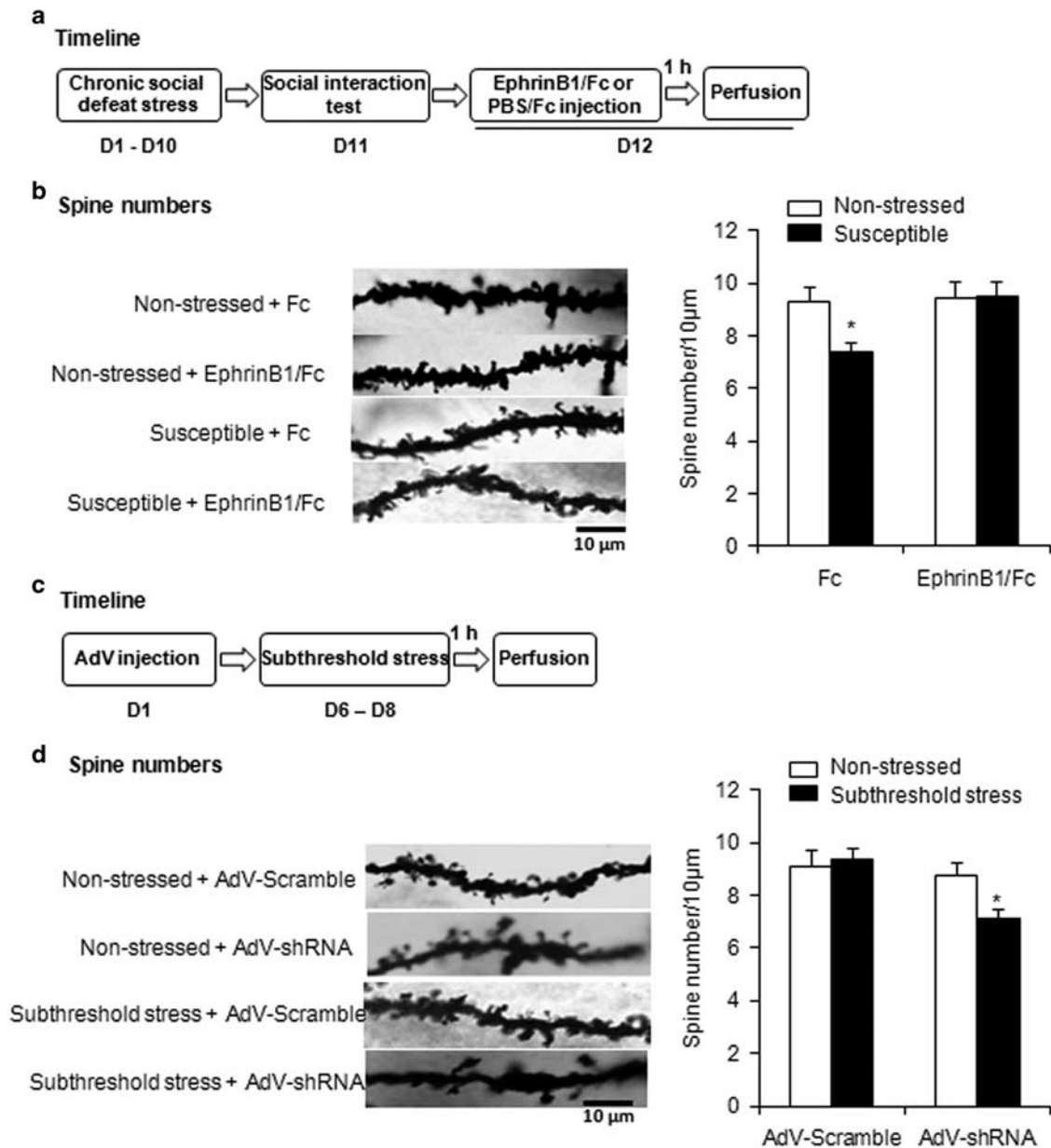


Figure 7 EphB2 influenced the number of dendritic spines in the mPFC. (a) Experimental timeline of chronic social defeat stress and EphrinB1/Fc microinjection. (b) Representative photograph of Golgi–Cox staining and total dendritic spine density under different conditions. * $p < 0.05$, compared with the non-stressed Fc group. $n = 3–5$ per group. Thirty neurons per group were analyzed and three dendritic segments of each neuron were selected for the analysis. (c) Experimental timeline of adenovirus microinjection and subthreshold chronic social defeat stress. (d) Representative photograph of Golgi–Cox staining and total dendritic spine density under different conditions. * $p < 0.05$, compared with the subthreshold stress AdV_{Scramble} group. $n = 3–5$ per group. Thirty neurons were analyzed per group, and three dendritic segments of each neuron were selected for the analysis.

of stress-related disorders (Arnsten, 2009). Previous studies showed that the optogenetic activation of mPFC in susceptible mice reversed depressive-like behavior after chronic social defeat stress (Covington *et al*, 2010; Vialou *et al*, 2014). The application of *N*-methyl-D-aspartate (NMDA) receptor antagonists produced antidepressant-like effects by increasing synaptic plasticity and glutamatergic transmission in the mPFC (Miller *et al*, 2014; Zhang *et al*, 2015; Zhu *et al*, 2013). The OFC is adjacent to the mPFC. The role of the OFC in the stress response is controversial. Previous studies showed that spine density in the OFC was increased after 21 days of repeated restraint stress (Liston

et al, 2006), whereas stress during development caused a loss of spines in the OFC (Muhammad *et al*, 2012). In the present study, we found that chronic social defeat stress decreased the expression of EphB2 and downstream proteins in the mPFC but not in the OFC in susceptible mice. Furthermore, the modulation of EphB2 signaling in the mPFC but not in the OFC regulated the vulnerability to stress. These results indicate that the effects of EphB2 on stress vulnerability were anatomically specific.

The tyrosine kinase receptor EphB2 has an important role in regulating neuronal development and maturation, GluR function, and synaptic plasticity. EphB2 is enriched at

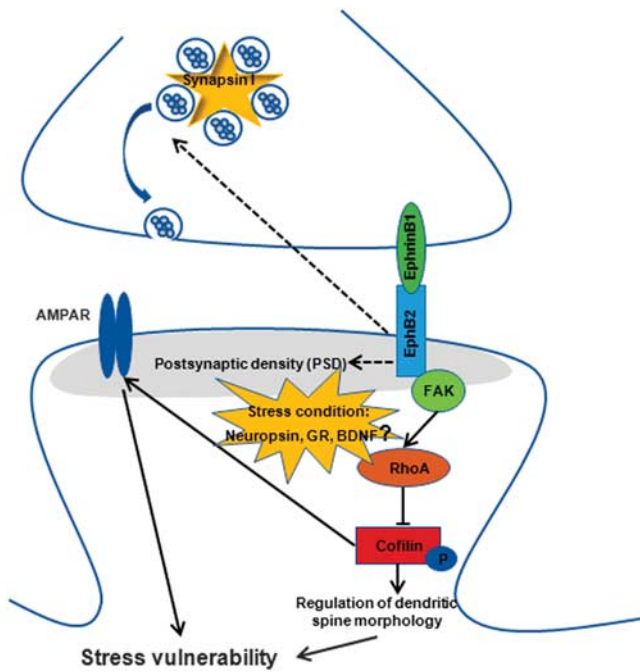


Figure 8 Proposed model of the possible signaling cascade of EphB2 that regulates stress vulnerability. EphB2 has been previously shown to inactivate cofilin through the FAK-dependent activation of RhoA, which maintains the morphology of mature spines. Chronic social defeat stress decreased the expression of EphB2 and some synaptic proteins and the phosphorylation of cofilin in the mPFC in susceptible mice. EphB2 may regulate stress vulnerability by modulating spine remodeling and synaptic plasticity via cofilin and AMPA receptor trafficking in the mPFC. Under subthreshold stress conditions, other stress-sensitive molecules, such as neuropsin, glucocorticoid receptors (GRs), brain-derived neurotrophic factor (BDNF), and corticotropin-releasing factor receptors, may interact with EphB2 to regulate spine structure and ultimately depressive-like behavior.

excitatory synapses in the mature brain and is involved in synapse and spine formation and maintenance (Aoto and Chen, 2007). EphB2-deficient mice exhibited impairments in synaptic transmission and plasticity (Simon *et al*, 2009) and a decrease in the mobility of postsynaptic dendritic filopodia during synapse formation *in vitro* (Kaiser *et al*, 2008). EphB2 also has an important role in dendritic spine morphogenesis in hippocampal neurons, which may be mediated by the clustering of endogenous syndecan-2 or actin polymerization through Rho-family guanosine triphosphatases (Ethell *et al*, 2001; Irie and Yamaguchi, 2002; Shi *et al*, 2009). Previous studies showed that the improper expression of EphB2 contributes to central nervous system diseases. A reduction of hippocampal EphB2 was associated with impaired cognitive function in a mouse model of Alzheimer's disease (Cisse *et al*, 2011; Simon *et al*, 2009), whereas EphB2 blockade in the amygdala hindered the development of restraint stress-induced anxiety (Attwood *et al*, 2011). EphB2 also has a role in emotional learning and memory (Cruz *et al*, 2015; Dines *et al*, 2015; Trabalza *et al*, 2012). In the present study, we found that EphB2 activation in the mPFC promoted resilience to stress, whereas EphB2 knockdown in the mPFC induced depressive-like behaviors in the subthreshold social defeat stress paradigm. Our results demonstrate that manipulating EphB2 signaling alters the vulnerability to stress.

Cofilin is a crucial regulator of actin dynamics and has important roles in dendritic spine remodeling, synaptic plasticity, and ultimately behavior (Rust, 2015). Cofilin has been implicated in the pathogenesis of neurodegenerative diseases (Garvalov *et al*, 2007; Minamide *et al*, 2000), the formation and maintenance of memories (Kramer *et al*, 2013; Wang *et al*, 2013), and stress-induced spine loss (Castaneda *et al*, 2015). Cofilin activity is regulated by phosphorylation. Previous studies showed that EphB2 activation suppressed cofilin activity by LIMK-mediated phosphorylation (Shi *et al*, 2009), which is consistent with our findings, in which EphB2 activation with EphrinB1-Fc reversed the decrease in p-cofilin that was induced by chronic social defeat stress. Cofilin inactivation has been reported to increase the number of mature spines with large heads (Rust *et al*, 2010; Shi *et al*, 2009) and protect neurons against amyloid β -induced spine loss (Davis *et al*, 2011; Shankar *et al*, 2007). In the present study, we found that EphB2 activation increased the total spine density in the mPFC in susceptible mice, which may be mediated by cofilin phosphorylation. Once dephosphorylated, activated cofilin can conjunct with actin fibers and induce depolymerization of the cytoskeleton, which induces the shrinkage of dendritic spines (Tomasella *et al*, 2014). Cofilin activation by dephosphorylation leads to the destabilization and transformation of mature mushroom spines into immature thin spines in hippocampal neurons (Shi and Ethell, 2006; Shi *et al*, 2009). Therefore, EphB2 knockdown decreased the total spine density likely through cofilin dephosphorylation. Alterations in the density and organization of spines in the mPFC are thought to contribute to the behavioral symptoms of depression (Duman and Duman, 2015). Altogether, this signaling cascade connects extracellular stimuli with dynamic reorganization of the actin cytoskeleton, which ultimately contributes to spine remodeling and depression-related behavior.

Previous studies showed that EphB2 controls the surface trafficking of GluR1 and GluR2 in neurons and has been implicated in cognitive disorders (Hussain *et al*, 2015; Miyamoto *et al*, 2016). We also found that EphB2 modulation in the mPFC influenced the expression of membrane GluR1 and GluR2. Moreover, cofilin was shown to regulate AMPA receptor trafficking and synaptic plasticity (Gu *et al*, 2010; Mizuno, 2013; Shen *et al*, 2009). Increases in p-cofilin levels are accompanied by additional AMPA receptor trafficking during long-term potentiation (Wang *et al*, 2013), whereas active ADF (actin depolymerizing factor)/cofilin is needed for spine pruning during long-term depression (Zhou *et al*, 2004). The synaptic insertion or removal of AMPA receptors regulates synaptic strength and participates in behavioral modification (Kessels and Malinow, 2009). The enhancement of AMPA receptor function produces antidepressant-like effects (Alt *et al*, 2005; Andreassen *et al*, 2015; Farley *et al*, 2010) and mediates the response to antidepressant treatment (Zhang *et al*, 2013). AMPAR regulation is also linked to individual variations in stress vulnerability (Schmidt *et al*, 2010; Vialou *et al*, 2010). In the present study, we found that chronic social defeat stress significantly reduced total spine density in the mPFC in susceptible mice, accompanied by a decrease in the expression of GluR1 and GluR2, suggesting that glutamatergic synaptic transmission was impaired in the mPFC in

susceptible mice, which is consistent with previous studies (Vialou *et al*, 2014; Yuen *et al*, 2012). Altogether, we speculate that EphB2 influences stress vulnerability by modulating spine remodeling and synaptic plasticity via cofilin and AMPA receptor trafficking in the mPFC.

However, conclusions that are drawn with regard to the causal role of EphB2 in the regulation of cofilin phosphorylation and AMPA receptor trafficking during social defeat stress should be made with caution. EphB2 knockdown reduced the phosphorylation of cofilin and the expression of membrane GluR1 and GluR2 only under the condition of subthreshold stress, indicating that additional signaling mechanisms that are engaged by subthreshold stress appear to converge with EphB2 to drive the changes in downstream signals and behavioral outcomes. Indeed, restraint stress induced the neuropsin-dependent cleavage of EphB2 in the amygdala and triggered its dissociation from NR1, resulting in modulation of the expression of NMDA receptor-dependent genes (Attwood *et al*, 2011). Additionally, under conditions of stress, glucocorticoid receptors (GRs) may translocate to the nucleus and promote local gene transcription. Alternatively, GRs may translocate to synapses and promote the LIMK1-dependent phosphorylation of cofilin (van der Kooij *et al*, 2016). Chronic stressor exposure can desensitize GRs, diminish their transcriptional activity, and produce detrimental effects (Sousa *et al*, 2008). Stress-regulated cofilin was also found to depend on RAC-1 signaling (Golden *et al*, 2013). Other stress-sensitive molecules, such as brain-derived neurotrophic factor and corticotropin-releasing factor receptors, may interact with EphB2 to regulate spine structure and ultimately depressive-like behavior.

We also evaluated the effects of stress and EphB2 on the levels of other synaptic proteins, including synapsin I and PSD95, in the mPFC. We found that EphrinB1/Fc reversed the decreases in synapsin I and PSD95 that were induced by chronic social defeat stress, whereas EphB2 knockdown decreased the levels of synapsin I and PSD95 in the subthreshold defeat stress paradigm, suggesting that EphB2 may regulate structural and synaptic plasticity in both a presynaptic and postsynaptic manner.

In conclusion, our results indicate that EphB2 activation in the mPFC produces rapid and prolonged stress-resistant and antidepressant-like effects by regulating spine density. These findings provide further insights into the molecular mechanisms that underlie stress resilience. Targeting EphB2 signaling may be considered a practical therapeutic strategy for depression.

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Author contributions

RZ, YH, WZ, and LL conceived the project and designed the experiments. RZ, LX, JL, NC, and CS performed the experiments. RZ, YH, and WZ analyzed the data. RZ, CC, YH, and LL wrote the paper with contributions from all of the other authors.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)