

α_1 - and β_3 -Adrenergic Receptor-Mediated Mesolimbic Homeostatic Plasticity Confers Resilience to Social Stress in Susceptible Mice

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ABSTRACT

BACKGROUND: Homeostatic plasticity in mesolimbic dopamine (DA) neurons plays an essential role in mediating resilience to social stress. Recent evidence implicates an association between stress resilience and projections from the locus coeruleus (LC) to the ventral tegmental area (VTA) (LC→VTA) DA system. However, the precise circuitry and molecular mechanisms of the homeostatic plasticity in mesolimbic DA neurons mediated by the LC→VTA circuitry, and its role in conferring resilience to social defeat stress, have not been described.

METHODS: In a well-established chronic social defeat stress model of depression, using projection-specific electrophysiological recordings and optogenetic, pharmacological, and molecular profiling techniques, we investigated the functional role and molecular basis of an LC→VTA circuit in conferring resilience to social defeat stress.

RESULTS: We found that LC neurons projecting to the VTA exhibit enhanced firing activity in resilient, but not susceptible, mice. Optogenetically mimicking this firing adaptation in susceptible mice reverses their depression-related behaviors, and induces reversal of cellular hyperactivity and homeostatic plasticity in VTA DA neurons projecting to the nucleus accumbens. Circuit-specific molecular profiling studies reveal that α_1 - and β_3 -adrenergic receptors are highly expressed in VTA→nucleus accumbens DA neurons. Pharmacologically activating these receptors induces similar proresilient effects at the ion channel and cellular and behavioral levels, whereas antagonizing these receptors blocks the proresilient effect of optogenetic activation of LC→VTA circuit neurons in susceptible mice.

CONCLUSIONS: These findings reveal a key role of the LC→VTA circuit in mediating homeostatic plasticity in stress resilience and reveal α_1 - and β_3 -adrenergic receptors as new molecular targets for therapeutically promoting resilience.

Keywords: Adrenergic receptors, Depression, Locus coeruleus, Nucleus accumbens, Resilience, Ventral tegmental area

<https://doi.org/10.1016/j.biopsych.2018.08.020>

Recently, a growing body of studies has begun to pay attention to how some individuals maintain seemingly unaltered psychophysiological functioning (resilient to depression), while others are more susceptible when they experience stress (1,2).

Resilience is defined as “the process of adapting well in the face of adversity, trauma, tragedy, threats or even significant sources of threat” (3). By achieving beneficial adaptations, resilient individuals are able to survive and even thrive despite exposure to high levels of adversity, such as prolonged severe stress (1,2,4,5). Investigating resilience mechanisms in the brain becomes important because such studies could provide a conceptually novel strategy to treat stress-related illnesses

by promoting mechanisms of natural resilience (2). However, in contrast to stress-induced pathological alterations, less is known about the neural mechanisms that underlie beneficial adaptations for resilience in the brain. Recent investigations demonstrate that resilient individuals actively use more genes to cope with stress and establish stable neural adaptations (5–7). Further, an increasing number of studies have begun to reveal the important neural mechanisms of such active resilience (1,8–12). More recent work has uncovered a physiological role of ventral tegmental area (VTA) to nucleus accumbens (NAc) (VTA→NAc) dopamine (DA) neurons in resilience versus susceptible behavioral phenotypes in the chronic social defeat

stress (CSDS) model of depression (10,11,13). Moreover, a homeostatic plasticity—a new balance between I_h current (hyperpolarization-activated cation channel current) and potassium (K^+) channel currents—in mesolimbic DA neurons mediates active resilience to CSDS (11). However, how upstream brain regions control these pathway-specific DA neurons to establish resilient adaptations and reverse depressive-like behaviors in susceptible animals remains unknown.

The locus coeruleus (LC), a norepinephrine (NE)-producing nucleus of the brainstem, is important for modulating vigilance (14), arousal (15), cognition (16), sleep/awake transitions (17), and drug addiction (18). The LC has also been implicated in stress resilience in both humans and rodent models (4,19,20). One of the key downstream brain regions of LC neurons is the VTA. Recently, it has been revealed that resilient mice had an increase in NE release from LC neurons that project to the VTA (21), implicating the role of NE in the VTA in mood regulation. In the present study, we examined whether LC→VTA circuit neurons mediate the homeostatic plasticity mechanisms in mesolimbic DA neurons and investigated which adrenergic receptors mediate this resilience plasticity.

METHODS AND MATERIALS

Animals

Male 7-week-old C57BL/6J (Jackson Laboratory, Bar Harbor, ME) and CD-1 retired breeders (Charles River, Wilmington, MA) were used to perform the CSDS paradigm. Ten-week-old DAT-IRES-Cre knock-in mice (22) were used to determine the gene expression of adrenergic receptors in projection-specific VTADA neurons using the circuit-mapping molecular profiling technique Retro-TRAP (23,24). All mice were singly housed on a 12-hour light/dark schedule, with food and water available ad libitum.

CSDS Paradigm

CSDS modeling was performed according to published protocols (6,10,11,13,25,26) and is described in the Supplement.

Social Interaction Test

Social interaction (SI) tests were performed on day 11 or following related pharmacological and optogenetic treatments, which were performed in the study as described in the Supplement and as described previously (6,10,11,25,26).

Ex Vivo Electrophysiology

Mice were anaesthetized with isoflurane and perfused immediately with ice-cold artificial cerebrospinal fluid, which contained 128-mM NaCl, 3-mM KCl, 1.25-mM NaH_2PO_4 , 10-mM D-glucose, 24-mM NaHCO_3 , 2-mM CaCl_2 , and 2-mM MgCl_2 (oxygenated with 95% O_2 and 5% CO_2 , pH 7.4, 295–305 mOsm). Cell-attached and whole-cell patch-clamp recording was performed in acute brain slices containing the LC or VTA as reported in our previous studies (10,11,25).

In Vivo Single-Unit Electrophysiological Recording

As we previously reported, mice were anesthetized with 10% chloral hydrate (400 mg/kg), and heads were fixed onto a stereotaxic frame horizontally (25). Using bregma, the LC was

located within the following ranges: anteroposterior: −5.30 to −5.50 mm; mediolateral: 0.50 to 1.20 mm; dorsoventral: −2.70 to −4.00 mm. Glass micropipettes (15–20 M Ω) filled with 2-M NaCl were used for recording. LC neurons were identified with criteria reported in previous studies (27,28).

Molecular Profiling

DAT-IRES-Cre mice were injected in the NAc with chicken anemia virus-green fluorescent protein (0.5 μL ; coordinates: ± 1.0 mm mediolateral, +1.35 mm anteroposterior, ± 4.2 mm dorsoventral), as well as in the VTA with adeno-associated virus (AAV)-IV-NBL10 (0.5 μL ; coordinates: ± 0.5 mm mediolateral, ± 3.15 mm anteroposterior, ± 4.2 mm dorsoventral). Fifteen days after injections, mice were sacrificed and the VTA was rapidly dissected on ice. Briefly, a 2-mm slice was made approximately covering the region 2 to 4 mm posterior to bregma. Brains were then pooled into three groups of 6 mice per group, homogenized in the presence of recombinant nanobody (100 ng/mL) (ChromoTek, Planegg, Germany), and centrifuged to clarify. Green fluorescent protein immunoprecipitation was performed with two mouse monoclonal antibodies [19C8 and 19F7 (29)] according to previous protocols (23,24). The resulting RNA was purified using the Absolutely RNA Nanoprep Kit (Agilent, Santa Clara, CA) and analyzed using an Agilent 2100 Bioanalyzer, followed by reverse transcription (QuantiTect; QIAGEN, Hilden, Germany) and TaqMan quantitative polymerase chain reaction (QIAGEN). Gene expression was normalized to large ribosomal protein gene 123 (rp123) (23).

Statistics

Data are presented as mean $\pm$ SEM. All analyses were performed with GraphPad Prism 7 software (GraphPad Software, La Jolla, CA). Normality of the data was statistically tested by the D'Agostino-Pearson omnibus normality test. Normally distributed data from multiple groups were compared with one-way analysis of variance with/without repeated factors, followed by a post hoc Bonferroni multiple comparison test when appropriate. Data that did not pass the normality test were analyzed by a nonparametric testing (Kruskal-Wallis test followed by Dunn's multiple comparisons test). Adrenergic receptor genes' expression ratio was compared with a paired Student *t* test. Statistical significance was set at $p < .05$.

RESULTS

LC→VTA NE Neurons Display Enhanced Firing Activity in Resilient Mice

Taking advantage of a well-established chronic (10-day) social defeat stress model of depression (6,10–12), we segregated susceptible and resilient mice based on their SI test behavior: susceptible mice display profound social avoidance, a behavior that was absent in resilient mice despite exposure to equivalent stress (Figure 1A, B; Supplemental Figures S1 and S2). Resilient mice also lack other depressive behaviors that are displayed in susceptible mice (1,10,11,13). To assess possible changes in the firing activity of LC neurons, we carried out in vivo single-unit recordings from anesthetized stress-naïve control, susceptible, and resilient mice to measure both firing rate and bursting (phasic) properties (30) (Figure 1C, D). We observed

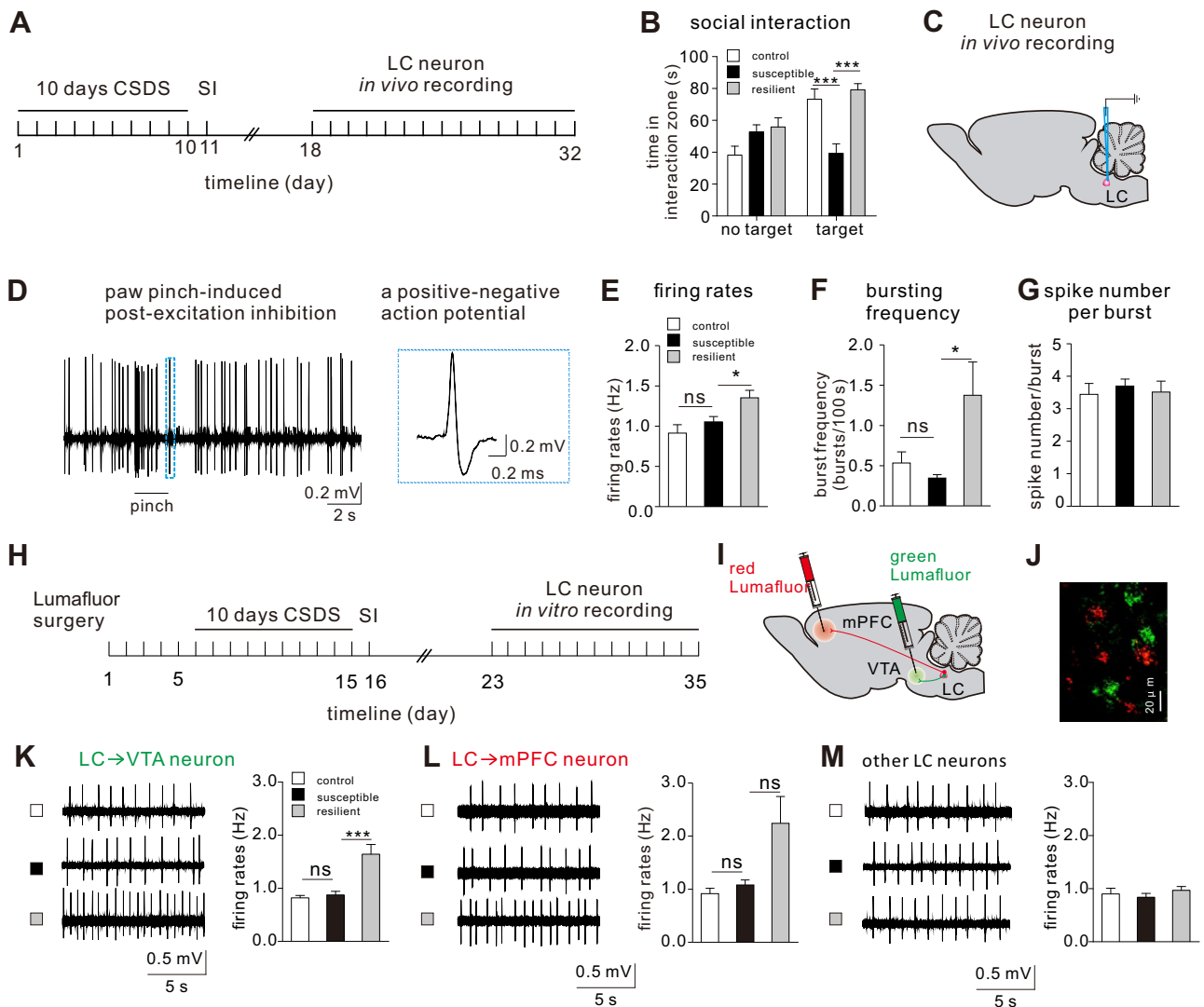


Figure 1. Locus coeruleus (LC) to ventral tegmental area (VTA) (LC → VTA) circuit neurons display increased firing activity selectively in resilient mice. **(A)** Experimental timeline of chronic social defeat stress (CSDS), social interaction (SI), and in vivo single-unit recording experiments. **(B)** SI measures on day 11 showing the significantly decreased social interaction time in susceptible mice (two-way analysis of variance [ANOVA] [$F_{2,52} = 11.19$, $p < .0001$], post hoc Bonferroni test [control vs. susceptible, *** $p = .0002$; susceptible vs. resilient, *** $p < .0001$], $n = 9$ –10 mice per group). **(C)** Schematic showing in vivo single-unit recordings. **(D)** Sample traces showing foot pinch-induced postexcitation inhibition and a typical action potential of an LC norepinephrine neuron. **(E)** Increased firing rates of LC neurons in the resilient mice (one-way ANOVA [$F_{2,91} = 6.38$, $p = .0026$], post hoc Bonferroni test [control vs. susceptible, $p = .91$; susceptible vs. resilient, * $p = .0342$], $n = 21$ –39 cells/6–8 mice per group). **(F)** Increased burst frequencies/100 seconds of LC neurons in the resilient mice (one-way ANOVA [$F_{2,79} = 3.94$, $p = .0233$], post hoc Bonferroni test: control vs. susceptible, $p > .9999$; susceptible vs. resilient, * $p = .026$, $n = 17$ –33 cells/6–8 mice per group). **(G)** No significant difference was observed in spike number per burst between groups (Dunn's multiple comparisons test [control vs. susceptible, $p > .9999$; susceptible vs. resilient, $p = .4969$], $n = 17$ –33 cells/6–8 mice per group). **(H)** Experimental timeline of ex vivo pathway-specific cell-attached recordings. **(I)** Schematic showing retrobeams (Lumafluors) injected into the medial prefrontal cortex (mPFC) and VTA. **(J)** Retrobean-labeled LC neurons (green: LC → VTA neurons; red: LC → mPFC neurons). **(K)** Sample traces of neuronal firing, and quantitative data showing the significantly increased LC → VTA neuron firing rates of the resilient mice (one-way ANOVA [$F_{2,40} = 13.46$, $p < .0001$], post hoc Bonferroni test [control vs. susceptible, $p > .9999$; susceptible vs. resilient, $p < .0001$], $n = 8$ –20 cells/4 mice per group). **(L)** Sample traces of neuronal firing, and quantitative data showing no difference in LC → mPFC neuron firing rates between groups (Dunn's multiple comparisons test [control vs. susceptible, $p = .7882$; susceptible vs. resilient, $p = .2257$], $n = 10$ –23 cells/4 mice per group). **(M)** Sample traces of neuronal firing, and quantitative data showing no difference in unlabeled LC neuron firing rates between groups (Dunn's multiple comparisons test [control vs. susceptible, $p > .9999$; susceptible vs. resilient, $p > .9999$], $n = 9$ –23 cells/4 mice per group). Error bars indicate mean $\pm$ SEM. ns, no significance.

that the in vivo firing rate and bursting frequency (per 100 seconds) of LC neurons were significantly increased in the resilient mice when compared with control and susceptible mice, with no change observed in spike number per burst event

(Figure 1E–G). There was also a trend for an increase in the percentage of spikes found in a burst and time spent bursting in the resilient mice as compared with control and susceptible mice (Supplementary Figure S3).

Next, we tested whether the firing changes observed in the LC neurons of resilient mice have projection-target specificity. To label specific projections and measure the baseline firing of these circuit neurons in an ex vivo slice preparation (18,31,32), we injected a green retrobean (Lumafluor Corp., Durham, NC) into the VTA and a red retrobean (Lumafluor) into the medial prefrontal cortex (mPFC) (Figure 1H–J). These two brain regions are well known to contribute to the segregation of the susceptible and resilience phenotypes, respectively (6,9,11,33,34). Interestingly, we found that only 3.5% of LC neurons colabeled with both red and green retrobeans (Supplemental Figure S4A, B), suggesting that the majority of LC→VTA and LC→mPFC projection neurons are distinct subpopulations. Furthermore, our ex vivo slice recordings show that LC→VTA neurons (green, ~98% are tyrosine hydroxylase positive) (Supplemental Figure S4C, D) exhibited increased firing in resilient mice when compared with control and susceptible groups, with no difference observed for firing frequencies in LC→mPFC neurons (Figure 1K, L). Unexpectedly, unlabeled LC neurons displayed firing rates comparable to control neurons (Figure 1M), suggesting that firing alterations may be specific to LC→VTA projecting neurons.

We subsequently focused on the LC→VTA projection pathway because of its crucial role in segregating susceptible and resilient phenotypes (6). Moreover, our previous work demonstrated signature adaptations in VTA DA neurons

projecting to the NAc that drive resilience (11). Therefore, we hypothesized that the increased firing adaptation in the LC→VTA circuit may contribute significantly to resilience mechanisms observed in the VTA.

Repeated Optical Activation of LC→VTA Neurons Promotes Resilience in Susceptible Mice

To investigate the relationship between the firing alterations of LC→VTA neurons and behavioral outcomes, we utilized a combination of viral and optogenetic techniques (11,13). To specifically target the LC→VTA pathway, we injected a retrograde AAV2/5-Cre into the VTA, and Cre-inducible AAV5-double floxed (DIO)-channelrhodopsin-2 (ChR2)-enhanced yellow fluorescent protein (eYFP) into the LC to selectively express ChR2 in LC→VTA neurons (AAV5-DIO-eYFP as control virus) (Figure 2A, B). We confirmed that optical phasic stimulation (five pulses/10-ms pulse width) of ex vivo LC→VTA neurons reliably induced five spikes and optocurrents in whole-cell current- and voltage-clamp modes, respectively (Supplemental Figure S5A, B). With this confirmation, we performed these dual injection surgeries before the onset of chronic social defeat stress. Following the defeat paradigm and the first SI test, susceptible and stress-naïve control mice were implanted with an optical fiber (ferrule) placed above the LC for selective stimulation of ChR2-

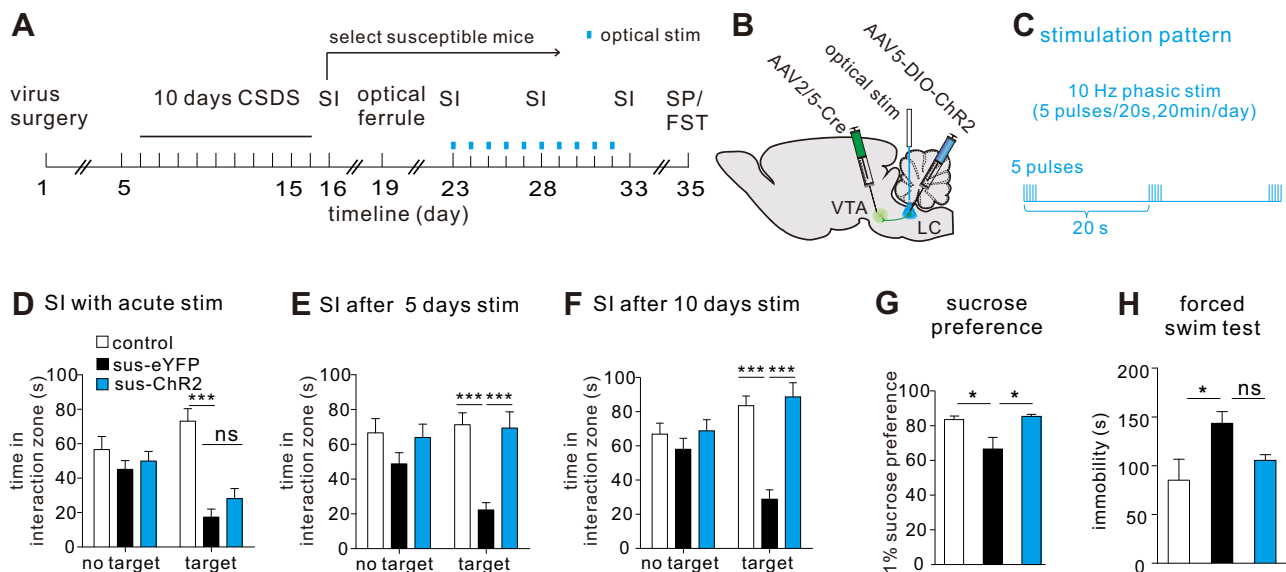


Figure 2. Repeated optogenetic activation of locus coeruleus (LC) to ventral tegmental area (VTA) (LC→VTA) neurons promotes resilience in previously defined susceptible mice. **(A)** Experimental timeline of surgeries, chronic social defeat stress (CSDS), social interaction (SI), optical stimulation, sucrose preference (SP), and forced swim test (FST). **(B)** Schematic showing viral surgeries and optical stimulation. **(C)** Optical phasic stimulation pattern. **(D)** No significant difference was observed in SI time during optical stimulation between the susceptible-enhanced yellow fluorescent protein (sus-eYFP) group and susceptible-channelrhodopsin-2 (sus-ChR2) group (two-way analysis of variance [ANOVA] [$F_{2,124} = 6.562, p = .002$], post hoc Bonferroni test [control vs. sus-eYFP, $***p < .0001$; sus-eYFP vs. sus-ChR2, $p > .9999$], $n = 21$ – 23 mice per group). **(E)** Increased SI time after 5 days of repeated optical stimulation in sus-ChR2 group (two-way ANOVA [$F_{2,122} = 3.114, p = .0479$], post hoc Bonferroni test [control vs. sus-eYFP, $***p < .0001$; sus-eYFP vs. sus-ChR2, $***p < .0001$], $n = 20$ – 23 mice per group). **(F)** Increased SI time after 10 days of repeated optical stimulation in the sus-ChR2 group (two-way ANOVA [$F_{2,124} = 8.696, p = .0003$], post hoc Bonferroni test [control vs. sus-eYFP, $***p < .0001$; sus-eYFP vs. sus-ChR2, $***p < .0001$], $n = 21$ – 23 mice per group). **(G)** Increased SP after 10 days of repeated optical stimulation in sus-ChR2 group (one-way ANOVA [$F_{2,18} = 6.29, p = .0085$], post hoc Bonferroni test [control vs. sus-eYFP, $*p = .0381$; sus-eYFP vs. sus-ChR2, $*p = .012$], $n = 6$ – 8 mice per group). **(H)** No significant changes were observed in immobility time in the FST after 10 days of repeated optical stimulation between groups (Dunn's multiple comparisons test [control vs. sus-eYFP, $*p = .0340$; sus-eYFP vs. sus-ChR2, $p = .0563$], $n = 5$ – 8 mice per group). Error bars indicate mean $\pm$ SEM. AAV, adeno-associated virus; DIO, double floxed; ns, no significance.

expressing LC→VTA neurons (Supplemental Figure S6). After recovery, we delivered in vivo optical phasic stimulation to LC→VTA neurons for 5 minutes during a second SI test and failed to observe significant effects on social avoidance behavior (Figure 2D; Supplemental Figure S7). We then turned to chronic stimulations to determine if repeated activation (20 minutes/day for 5 or 10 days) of the LC→VTA circuit could reverse depressive-like behaviors (Figure 2A–C). Our data show that eYFP-expressing susceptible mice continue to display profound and stable avoidant behavior when compared with control mice (Figure 2E, F), and to themselves across repeated SI tests (for time in interaction zone, two-way analysis of variance followed by post hoc Bonferroni test [target: SI^{Figure 2D} vs. SI^{Figure 2E}, $p > .9999$; SI^{Figure 2D} vs. SI^{Figure 2F}, $p = .4200$; SI^{Figure 2E} vs. SI^{Figure 2F}, $p > .9999$]). However, the avoidant behaviors of ChR2-expressing susceptible mice were reversed following 5 days (Figure 2E; Supplemental Figure S8A) and 10 days (Figure 2F; Supplemental Figure S9A) of LC→VTA stimulation, without affecting locomotor activity during the SI tests (Supplemental Figures S8B, C and S9B, C). The 10-day optical stimulation of LC→VTA neurons also significantly increased sucrose preference and tended to decrease immobility in a forced swim test (Figure 2G, H; Supplemental Figure S10), further demonstrating proresilient effects. These results support the view that repeated activation of the LC→VTA circuit promotes resilience and beneficially reverses behavioral abnormalities in otherwise susceptible mice.

Repeated Optical Activation of LC→VTA Neurons in Susceptible Mice Enhances the Firing Activity of LC Neurons

To further investigate the cellular mechanisms that underlie the proresilient effects following 10-day optical stimulation of LC→VTA circuit, we examined alterations in LC neurons and their downstream targets in VTA DA neurons. We first

confirmed the behavioral phenotypes in a new cohort of socially defeated mice (Supplemental Figure S11), then carried out in vivo single-unit recordings from LC neurons (continuing the stimulation 20 min/day until the day of in vivo recordings, as shown in Figure 3A, B). Our data demonstrate that repeated optical stimulation of LC→VTA neurons increased the in vivo firing rate and bursting frequency of LC neurons (when compared with the absence of optical stimulation), with no change observed in spike number per bursting event, when compared with control neurons (Figure 3C–E). There is a significant increase or a trend of increase in percentages of bursting spikes and time in burst in the optically stimulated group as compared with the control group (Supplemental Figure S12). These results induced by repeated LC→VTA optical activation are similar to the in vivo recordings observed in naturally resilient mice.

Repeated Optical Activation of LC→VTA Neurons in Susceptible Mice Induces Homeostatic Plasticity in VTA→NAc DA Neurons

To examine whether repeated optical stimulation of LC→VTA circuit induces downstream changes in the VTA, we focused on VTA DA neurons projecting to the NAc, the subpopulation of these neurons that display signature homeostatic adaptations in I_h current and K^+ channel currents in resilient mice and that mediate behavioral resilience (11). These channels establish a critical intrinsic balance to maintain control levels of firing in the VTA→NAc DA neurons of resilient mice as compared with the pathological increase in firing seen in susceptible mice (11). To test whether repeated optical stimulation of the LC→VTA circuit has an effect on the firing rate and balance in intrinsic currents balance of VTA→NAc DA neurons, we labeled VTA→NAc neurons by injecting red retrobeams into the NAc, and expressed ChR2 selectively in LC→VTA neurons (Figure 4A, B). We delivered 10-day in vivo

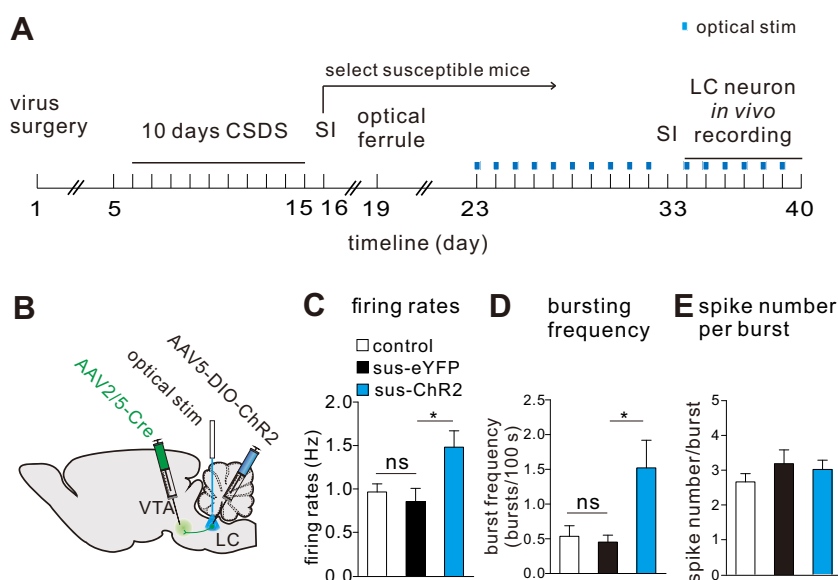


Figure 3. Repeated optical activation of locus coeruleus (LC) to ventral tegmental area (VTA) (LC→VTA) neurons in susceptible mice enhances the firing activity of LC neurons. **(A)** Experimental timeline for in vivo single-unit recordings following repeated stimulation of LC→VTA neurons. **(B)** Schematic showing surgeries for virus injections and optical stimulation of LC→VTA neurons. **(C)** Increased LC neuron firing rates after 10 days of repeated optical stimulation in the susceptible-channelrhodopsin-2 (sus-ChR2) group (one-way analysis of variance [$F_{2,84} = 4.82$, $p = .0104$], post hoc Bonferroni test [control vs. susceptible-enhanced yellow fluorescent protein (sus-eYFP), $p > .9999$; sus-eYFP vs. sus-ChR2, $p = .0484$], $n = 14$ –41 cells/4–7 mice per group). **(D)** Increased LC neuron bursting frequencies/100 seconds after 10 days of repeated optical stimulation in the sus-ChR2 group (one-way analysis of variance [$F_{2,40} = 5.06$, $p = .011$], post hoc Bonferroni test [control vs. sus-eYFP, $p > .999$; sus-eYFP vs. sus-ChR2, $p = .0433$], $n = 9$ –20 cells/4–7 mice per group). **(E)** No difference was observed in LC neuron spike number per burst between groups (one-way analysis of variance [$F_{2,40} = 0.90$, $p = .4154$], post hoc Bonferroni test [control vs. sus-

eYFP, $p = .6921$; sus-eYFP vs. sus-ChR2, $p > .9999$], $n = 9$ –20 cells/4–7 mice per group). Error bars indicate mean $\pm$ SEM. AAV, adeno-associated virus; CSDS, chronic social defeat stress; DIO, double floxed; SI, social interaction.

Mesolimbic α_1 - and β_3 -Adrenergic Receptors in Resilience

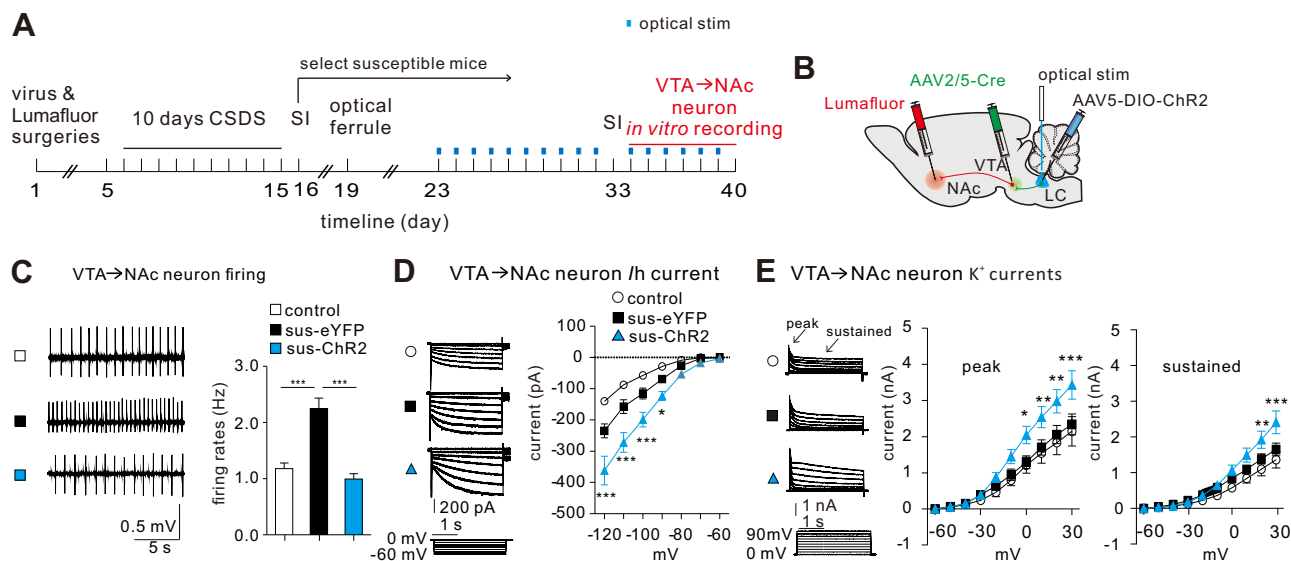


Figure 4. Repeated optical activation of locus coeruleus (LC) to ventral tegmental area (VTA) (LC→VTA) neurons in susceptible mice induces homeostatic plasticity in VTA→nucleus accumbens (NAc) dopamine neurons. **(A)** Experimental timeline. **(B)** Schematic showing surgeries for retrobean (Lumafluor) and virus injections, and optical stimulation of LC→VTA neurons. **(C)** Sample traces of neuronal firing, and quantitative data showing the significantly decreased VTA→NAc neuron firing rates after 10 days of repeated optical stimulation in the susceptible-channelrhodopsin-2 (sus-ChR2) group (one-way analysis of variance [ANOVA] [$F_{2,39} = 26.39, p < .0001$], post hoc Bonferroni test [control vs. susceptible-enhanced yellow fluorescent protein (sus-eYFP), $***p < .0001$; sus-eYFP vs. sus-ChR2, $***p < .0001$], $n = 12$ –17 cells/6–8 mice). **(D)** I_h current sample traces, and quantitative data showing the increased I_h currents in VTA→NAc neurons after 10 days of repeated optical stimulation in the sus-ChR2 group (two-way ANOVA [$F_{12,231} = 9.04, p < .001$], post hoc Bonferroni test [sus-eYFP vs. sus-ChR2, at -120 mV, $***p < .0001$; at -110 mV, $***p < .0001$; at -100 mV, $***p = .0003$; at -90 mV, $*p = .03$], $n = 9$ –15 cells/6–8 mice per group). **(E)** Potassium (K^+) currents sample traces, and quantitative data showing increased K^+ currents in VTA→NAc neurons after 10 days of repeated optical stimulation in the sus-ChR2 group (peak: two-way ANOVA [$F_{18,289} = 1.98, p < .0001$], post hoc Bonferroni test [sus-eYFP vs. sus-ChR2, at 30 mV, $***p = .0003$; at 20 mV, $**p = .0026$; at 10 mV, $**p = .0073$; at 0 mV, $*p = .0203$], $n = 9$ –13 cells/6–8 mice per group; sustained: two-way ANOVA [$F_{18,289} = 2.62, p < .0001$], post hoc Bonferroni test [sus-eYFP vs. sus-ChR2, at 30 mV, $***p < .0001$; at 20 mV, $**p = .0059$], $n = 9$ –13 cells/6–8 mice per group). Error bars indicate mean $\pm$ SEM. AAV, adeno-associated virus; CSDS, chronic social defeat stress; DIO, double floxed; SI, social interaction.

optical stimulation to LC→VTA neurons in the LC, as stated above, to reverse social avoidance (Supplemental Figure S13), and measured the firing rates and ionic currents from VTA→NAc putative DA neurons (large I_h current and >1.1 -ms triphasic waveform) (Supplemental Figure S14) of control, susceptible-eYFP, and susceptible-ChR2 groups. Our data show that 10-day optical stimulation of LC→VTA neurons completely reversed the pathological hyperactivity observed in the susceptible-eYFP group (Figure 3C) and achieved an intrinsic currents balance similar to that observed in resilient mice (Figure 4D, E). These results demonstrate that mimicking the resilience-associated firing adaptation in the LC→VTA circuit by repeatedly activating this circuit's neurons induces key resilience adaptations in VTA→NAc neurons.

VTA α_1 - and β_3 -Adrenergic Receptors Promotes Resilience and Intrinsic Currents Balance in VTA→NAc DA Neurons in Susceptible Mice

Our next question was which adrenergic receptors in the VTA mediate these effects. To perform molecular profiling of adrenergic receptors in VTA→NAc DA neurons, we injected retrograde tracer chicken anemia virus-GFP into the NAc and Cre-inducible AAV-FLEX-NBL10 into the VTA of stress-naïve DAT-IRES-Cre mice (Figure 5A, B). Immunostaining validation

shows a substantial number of VTA→NAc DA neurons targeted by this approach (Supplemental Figure S15). Our profiling data revealed that, among adrenergic receptor subunits, α_1 and β_3 were most highly expressed in VTA→NAc DA neurons as compared with their expression levels in all VTA DA neurons (Figure 5C).

We further tested whether α_1 and β_3 receptors are involved in mediating the resilience behavior and intrinsic currents balance induced by LC→VTA activation in the VTA→NAc circuit. Following chronic social defeat stress, we injected a red retrobean into the NAc to label VTA→NAc neurons, and locally infused α_1 and β_3 receptor agonists (methoxamine HCl and CL316243) into the VTA for 10 days, and then performed an SI test to measure social avoidance behavior (Figure 5D, E). We observed that 10-day intra-VTA infusion of this cocktail completely reversed social avoidant behaviors in susceptible mice when compared with vehicle-infused susceptible mice (Figure 5F; Supplemental Figure S16). We then performed ex vivo recordings to determine the cellular alterations within red retrobean-labeled VTA→NAc neurons following chronic infusions. Repeated infusion of α_1 and β_3 receptor agonists reversed the pathological hyperactivity of VTA→NAc neurons that is associated with susceptibility (Figure 5G). Moreover, these infusions increased I_h and K^+ currents of retrobean-labeled VTA→NAc neurons as compared with susceptible-vehicle control neurons (Figure 5H, I), reflecting the balance

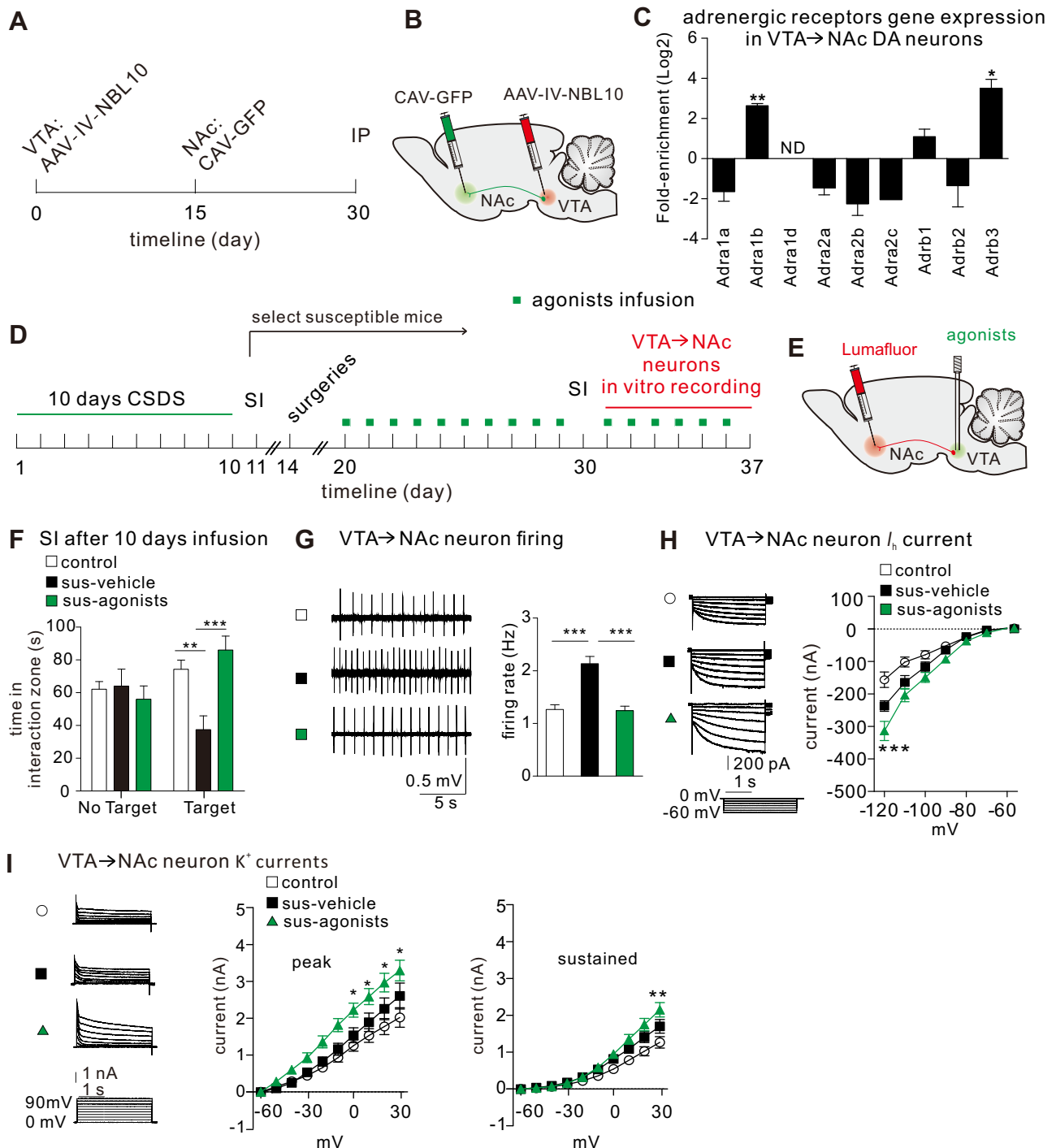


Figure 5. Activation of α_1 - and β_3 -adrenergic receptors is sufficient to induce resilience. **(A, B)** Experimental timeline and schematic for examination of ventral tegmental area (VTA) to nucleus accumbens (NAc) (VTA→NAc) pathway-specific adrenergic receptor genes. **(C)** Higher-fold enrichment of adrenergic receptors on VTA→NAc dopamine (DA) neurons (normalized to overall VTA DA neurons; enriched genes: *Adra1b* [$t_2 = 24.80$, $**p = .0016$] and *Adrb3* [$t_2 = 7.808$, $*p = .016$]; $n = 3$ groups/6 mice per group). **(D)** Experimental timeline. **(E)** Schematic showing agonists infusion and retrobead (Lumafluor) injection to label VTA→NAc neurons. **(F)** Increased social interaction (SI) time after 10 days of agonists infusion in susceptible (sus)-agonists group (two-way analysis of variance [ANOVA] [$F_{2,54} = 6.966$, $p = .002$], post hoc Bonferroni test [control vs. sus-vehicle, $**p = .0023$; sus-vehicle vs. sus-agonists, $***p = .0003$], $n = 9$ –12 mice per group). **(G)** Sample traces of neuronal firing, and quantitative data showing the significantly decreased VTA→NAc neuron firing rates after 10 days of agonists infusion in sus-agonists group (one-way ANOVA [$F_{2,63} = 22.72$, $***p < .0001$], post hoc Bonferroni test [control vs. sus-vehicle, $***p < .0001$; sus-vehicle vs. sus-agonists, $***p < .0001$], $n = 19$ –24 cells/9–12 mice per group). **(H)** I_h current sample traces, and quantitative data showing the increased I_h currents in VTA→NAc neurons after 10 days of agonists infusion in susceptible-channelrhodopsin-2 (sus-ChR2) group (two-way ANOVA [$F_{12,420} = 4.53$, $p < .0001$],

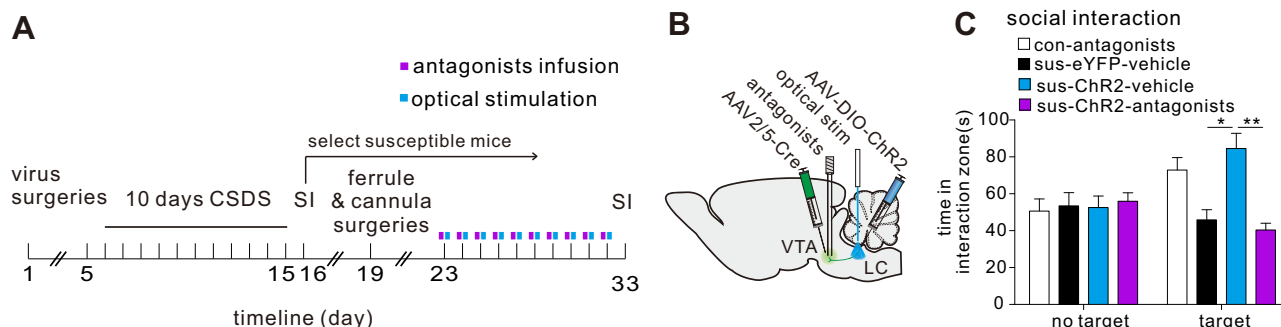


Figure 6. Ventral tegmental area (VTA) α_1 - and β_3 -adrenergic receptors are necessary to the proresilient effect of repeated optical stimulation. **(A)** Schematic showing virus surgeries, antagonist infusion, and optical stimulation. **(B)** Experimental timeline. **(C)** Increased social interaction (SI) time induced by repeated optical stimulation was blocked by 10 days of intra-VTA pretreatment with α_1 - and β_3 -adrenergic receptor antagonists (two-way analysis of variance [$F_{3,42} = 6.332$, $p = .0012$], post hoc Bonferroni test [susceptible-enhanced yellow fluorescent protein-vehicle (sus-eYFP-vehicle) vs. susceptible-channelrhodopsin-2-vehicle (sus-ChR2-vehicle), $*p = .0435$; sus-ChR2-vehicle vs. sus-ChR2-antagonists, $**p = .0157$], $n = 6$ –7 mice per group). Error bars indicate mean $\pm$ SEM. AAV, adeno-associated virus; CSDS, chronic social defeat stress; DIO, double floxed.

in intrinsic currents associated with natural resilience. These findings indicate that activation of these adrenergic receptors is sufficient to induce a resilience-like phenotype at the ion channel, cellular, and behavioral levels.

VTA α_1 - and β_3 -Adrenergic Receptors Are Necessary for the Proresilient Effect of Repeated Optical Stimulation of LC→VTA Neurons

To further study this mechanism, we investigated whether antagonists of α_1 and β_3 receptors block the proresilient effects induced by optical stimulation of LC→VTA neurons. We expressed ChR2 in LC→VTA neurons as stated above and delivered 10-day optical stimulation to LC→VTA neurons of susceptible mice with intra-VTA infusion of α_1 and β_3 antagonists (cyclazosin and SR59230A HCl) (Figure 6A, B). Such intra-VTA infusions (once a day for 10 days) completely blocked the optical stimulation-induced reversal of social avoidance behavior as compared with mice receiving vehicle (Figure 6C; Supplemental Figure S17), demonstrating that these receptors are necessary for the proresilient effects of optical stimulation of the LC→VTA circuit.

DISCUSSION

This study showed that 1) the firing activity of LC→VTA neurons is increased exclusively in resilient, but not susceptible, mice after CSDS modeling; 2) optogenetically mimicking this adaptive change (activating LC→VTA neurons) in stress-exposed susceptible mice promotes a resilience-like phenotype, including reversal of hyperactivity of VTA→NAc DA neurons, homeostatic plasticity (intrinsic balance between I_h current and K^+ currents), and normalization of social behavior; and 3) molecular profiling and pharmacological studies identify

α_1 - and β_3 -adrenergic receptors expressed by VTA DA neurons as being sufficient and necessary to induce resilience-like phenotypes. Overall, these circuit-specific investigations demonstrate that the LC→VTA→NAc pathway plays an important role in promoting resilience through norepinephrine mechanisms in the VTA.

We recently demonstrated that VTA→NAc DA neurons constitute a neural circuit in which a resilience-specific active form of homeostatic plasticity is established by an intrinsic balance of I_h current and K^+ currents to maintain normal neuronal activity as well as behavioral resilience (11). Following this finding, we further identified KCNQ-type K^+ channels as a target for conceptually novel antidepressants that function through the potentiation of active resilience mechanisms (35). Recently, researchers have started to investigate the extrinsic synaptic mechanisms that underlie this active homeostatic plasticity (21,36). It is well known that VTA DA neurons are regulated by intrinsic ion channels and by extrinsic synaptic innervation, including a heavy noradrenergic input from the LC (37–39). Our results in this study consistently support these early findings that the LC-NE system plays an important role in mediating resilience in human and animal models (4,19,21).

It is widely known that the LC-NE neurons respond to stress by globally priming neurons in the brain (4,31,40–45). Increasing evidence has also shows the heterogeneity of LC-NE neurons (45–48), as seen for VTA DA neurons (10,11,49,50). Here, our in vitro electrophysiological experiments showed that LC→VTA neurons fired significantly higher in the resilient subgroup as compared with stress-naïve control mice, while LC→VTA neurons of susceptible mice have a normal firing activity comparable to that of control mice. Our in vivo recording data further confirmed that LC neurons from resilient mice displayed enhanced firing rates and phasic firing

post hoc Bonferroni test [control vs. sus-vehicle, $***p = .0006$; sus-vehicle vs. sus-agonists, $***p = .005$], $n = 17$ –26 cells/9–12 mice per group). **(I)** Potassium (K^+) current sample traces, and quantitative data showing the increased K^+ currents in VTA→NAc neurons after 10 days of agonists infusion in the sus-ChR2 group (peak: two-way ANOVA [$F_{18,430} = 1.79$, $p = .0243$], post hoc Bonferroni test [sus-vehicle vs. sus-agonists, at 30 mV, $*p = .0154$; at 20 mV, $*p = .0113$; at 10 mV, $*p = .0141$; at -10 mV, $*p = .0235$], $n = 14$ –17 cells/9–12 mice per group; sustained: two-way ANOVA [$F_{18,430} = 3.62$, $p < .0001$], post hoc Bonferroni test [sus-vehicle vs. sus-agonists, at 30 mV, $**p = .0012$], $n = 14$ –17 cells/9–12 mice per group). Error bars indicate mean $\pm$ SEM. AAV, adeno-associated virus; CAV-GFP, chicken anemia virus-green fluorescent protein; CSDS, chronic social defeat stress; DIO, double floxed; IP, immunoprecipitation; IV, introverted; NBL10, N terminus of ribosomal subunit protein Rpl10a; ND, not detected in IP.

events as compared with control and susceptible mice, which is consistent with a recent study reporting greater NE release in the VTA of resilient mice (21). These findings indicated that hyperactivity of LC→VTA neurons might be an important hallmark of resilience in the brain. Hyperactivity of the LC-NE neurons has been implicated in mediating hyper-responsiveness, including increased vigilance, in post-traumatic stress disorder and arousal dysfunction (15,51). In our previous study using the CSDS model, we also observed elevated arousal in both susceptible and resilient mice (6), suggesting that increased arousal might be related to the development of stress susceptibility versus resilience depending on the timing of the vigilance. Greater vigilance before stressor exposure, as an active function, might promote resilience, whereas increased vigilance induced by stress, for example, hyperresponsiveness in posttraumatic stress disorder, would be passive and pathological. It would be very interesting to investigate the role for vigilance in stress resilience in future work, and it is possible that high levels of natural vigilance acts as a predictor of stress resilience.

Our *in vivo* optogenetic investigations confirmed that 5 or 10 days of repeatedly activating these neural circuit neurons was sufficient to reverse social avoidance in previously defined susceptible mice. Strikingly, this stimulation also induced homeostatic plasticity of VTA→NAc DA neurons, a featured self-tuning adaptive balance between I_h current and K^+ currents that is uniquely seen in resilient mice. In addition, this same stimulation consistently stabilized the firing activity and normalized social behavior. Interestingly, we observed that the firing variation in LC→mPFC circuit neurons is much greater than that in LC→VTA circuit neurons. We are not sure whether this indicates that different subgroups of LC→mPFC neurons play distinct functional roles in the response to social stress. In future work, it would be interesting to explore the functional role of other LC-related circuits, including LC→mPFC neurons, in mediating stress resilience versus susceptibility.

VTA DA neurons express multiple kinds of adrenergic receptors that might mediate the interaction between the LC-NE inputs and VTA-DA neurons. Taking advantage of a circuit- and cell type-specific molecular profiling technique (23), we identified enrichment of α_1 - and β_3 -adrenergic receptors in VTA→NAc DA neurons, and found that repeated pharmacological activation of VTA α_1 - and β_3 -adrenergic receptors with a cocktail of specific agonists reversed social avoidance behavior in previously identified susceptible mice. Strikingly, this treatment also completely normalized the pathological hyperactivity of VTA→NAc DA neurons and established a resilience-like intrinsic balance between I_h and K^+ currents, a critically important mechanism of homeostatic plasticity identified in naturally resilient mice and in susceptible animals after repeated optogenetic stimulation (11). These findings indicate that repeated activation of VTA α_1 - and β_3 -adrenergic receptors, and repeated activation of LC→VTA neurons, display similar proresilient effects in susceptible mice at the cellular and behavioral levels. This interesting phenomenon suggests that α_1 - and β_3 -adrenergic receptors might be key mediators of the synaptic relay between the LC-NE system and the VTA→NAc reward circuit. As expected, we observed that pretreatment with α_1 - and β_3 -receptor antagonists is sufficient to abolish the proresilience of repeated optogenetic activation

of LC→VTA neurons in the SI test. This study thereby establishes that α_1 - and β_3 -adrenergic receptors are necessary for the proresilient effects of LC-NE on VTA→NAc DA neurons. Another important finding of this study is that it introduces a novel extrinsic mechanism that accounts for the homeostatic plasticity in VTA→NAc DA neurons that is associated with resilience. In these studies, we combined pharmacological agonist and antagonist treatments for both adrenergic receptors to maximize our ability to detect their roles. Future studies are needed to examine whether manipulation of either receptor alone would be sufficient to induce antidepressant-like behavioral effects.

While the CSDS paradigm is a well-established and widely used rodent model of depression, our previous study (6) showed that socially defeated mice also exhibit anxiety-like phenotypes. In the present study, we reported that activation of LC→VTA circuit reversed deficits in social behavior and forced swim performance. It is not completely clear whether the reversal of these deficits was induced by normalizing depression- or anxiety-related components because SI and forced swim tests that we used in this study may include both components (52). Our results from the sucrose preference test suggest that depression components may contribute to the reversal effects observed. Nevertheless, the functional role of LC→VTA in the regulation of an anxiety phenotype in the context of CSDS needs to be explored in greater depth in the future.

Ketamine is used routinely for surgical anesthesia in rodents. There is now robust evidence that a single sub-anesthetic dose of ketamine produces rapid antidepressant effects in humans and animals (53,54). However, there has been no evidence so far showing that ketamine at anesthetic doses, such as 100 mg/kg in our case, which is far higher than used for depression treatment, has any antidepressant effect (6,10,11,13,25,35). Nevertheless, this caveat should be considered when interpreting data from animals that underwent ketamine-induced anesthesia.

Collectively, these observations provide highly consistent molecular, cellular, and behavioral evidence for the underlying mechanisms by which an LC→VTA→NAc circuit functions as a stress resilient pathway in the brain. Specifically, these results establish α_1 - and β_3 -adrenergic receptors as new molecular targets for therapeutically promoting natural resilience, a possibility that now warrants clinical testing, while current research focuses on obtaining a more complete understanding of the mechanisms of natural resilience.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by National Institute of Mental Health Grant Nos. R01 MH092306 (to M-HH) and R01 MH051399 (to EJN); National Natural Science Foundation of China Grant Nos. NSFC81230025, 81720108013 (to J-LC); NSFC81200862, 81771453 (to HZ); Jiangsu Province Natural Science Foundation Grant No. BK20171158 (to HZ); Xuzhou Medical University Start-up Grant for Excellent Talents Grant No. D2018010 (to HZ); National Research Service Award Grant Nos. F31 AA022862 (to BJ), T32 MH 087004 (to BJ), and F32 MH096464 (to AKF); a Johnson & Johnson/IMHRO Rising Star Translational Research Award (to M-HH); a NARSAD Independent Investigator Award (to M-HH); and Jiangsu Provincial Special Program of Medical Science Grant No. BL2014029 (to J-LC); and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

All authors report no biomedical financial interests or potential conflicts of interest.

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Received Feb 25, 2018; revised and accepted Aug 28, 2018.

Supplementary material cited in this article is available online at <https://doi.org/10.1016/j.biopsych.2018.08.020>.

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