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Essential Role of Mesolimbic Brain-Derived Neurotrophic Factor in Chronic Social Stress–Induced Depressive Behaviors

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ABSTRACT

BACKGROUND: Previous work has shown that chronic social defeat stress (CSDS) induces increased phasic firing of ventral tegmental area (VTA) dopamine (DA) neurons that project to the nucleus accumbens (NAc) selectively in mice that are susceptible to the deleterious effects of the stress. In addition, acute optogenetic phasic stimulation of these neurons promotes susceptibility in animals exposed to acute defeat stress. These findings are paradoxical, as increased DA signaling in NAc normally promotes motivation and reward, and the influence of chronic phasic VTA firing in the face of chronic stress is unknown.

METHODS: We used CSDS with repeated optogenetic activation and pharmacologic manipulations of the mesolimbic VTA-NAc pathway to examine the role of brain-derived neurotrophic factor (BDNF) and DA signaling in depressive-like behaviors. We measured BDNF protein expression and DA release in this model.

RESULTS: Pharmacologic blockade of BDNF–tyrosine receptor kinase B (TrkB) signaling, but not DA signaling, in NAc prevented CSDS-induced behavioral abnormalities. Chronic optogenetic phasic stimulation of the VTA-NAc circuit during CSDS exacerbated the defeat-induced behavioral symptoms, and these aggravated symptoms were also normalized by BDNF-TrkB blockade in NAc. The aggravated behavioral deficits induced by phasic stimulation of the VTA-NAc pathway were blocked as well by local knockdown of BDNF in VTA.

CONCLUSIONS: These findings show that BDNF-TrkB signaling, rather than DA signaling, in the VTA-NAc circuit is crucial for facilitating depressive-like outcomes after CSDS and they establish BDNF-TrkB signaling as a pathologic mechanism during periods of chronic stress.

Keywords: BDNF, Chronic defeat stress, Dopamine, Nucleus accumbens, Social avoidance, Ventral tegmental area

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Social stress is one of the most critical factors in the onset of depressive disorders in humans (1,2). The effect of social stress on depressive-like behavioral abnormalities has been investigated with the chronic social defeat stress (CSDS) paradigm in mice (3–5), in which susceptible and resilient phenotypes are segregated after 10 days of the stress. Depressive-like behaviors in susceptible mice have been causally associated with molecular and physiologic abnormalities in the mesolimbic dopamine (DA) pathway, which comprises the ventral tegmental area (VTA) and its projecting terminals to the nucleus accumbens (NAc) (3,4,6,7). For example, phasic, but not tonic, firing of VTA DA neurons is increased in susceptible, but not resilient, mice (4,6).

Brain-derived neurotrophic factor (BDNF) in the mesolimbic DA pathway has been implicated in the susceptible phenotype after CSDS (3,4). Elevated levels of BDNF protein expression in NAc are associated with depressive-like abnormalities induced by CSDS and are not observed in resilient mice (4). Localized *Bdnf* gene deletion in VTA of adult mice reduces susceptibility to CSDS (3), suggesting that BDNF, transported from VTA to NAc, induces behavioral susceptibility. In addition, the combination of 1 day of defeat plus acute optogenetic phasic stimulation of VTA-to-NAc DA neurons induces social avoidance and other deficits, whereas exposure to either 1-day defeat or acute optogenetic stimulation alone does not induce behavioral abnormalities in normal mice (7). Phasic stimulation of this pathway increases BDNF protein levels in the NAc of mice exposed to 1 day of defeat, and blockade of BDNF–tyrosine receptor kinase B (TrkB) signaling in NAc prevents the ability of acute optogenetic stimulation to induce behavioral deficits in this acute stress paradigm (8).

Phasic stimulation of the VTA-NAc pathway facilitates release of BDNF as well as DA from VTA DA terminals (9,10). Also, BDNF can facilitate DA release from DA terminals (11). A subset of VTA DA neurons has also been implicated in stress-elicited depressive-like abnormalities (12,13). Thus, it is conceivable that both DA and BDNF signaling in NAc might promote depressive phenotypes. However, this view is

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contrary to the established role of DA in mediating reward. In fact, DA deficiency in NAc has been postulated in depressed humans and animal models (14,15). Several clinical studies have shown that depressed patients have attenuated concentrations of DA metabolites (16–18). Moreover, optogenetic activation of VTA DA neurons reverses chronic mild stress-induced depression-associated behaviors in mice, whereas inhibition of these neurons promoted these behaviors, suggesting an antidepressant-like role of DA signaling (19). Finally, some antidepressants increase DA transmission in the NAc shell (20–22). The present study was designed to address these paradoxical findings. Our data establish that BDNF, but not DA, mediates the ability of a hyperactive VTA-NAc pathway to promote depressive-like symptoms in the CSDS paradigm.

METHODS AND MATERIALS

Experimental Subjects

Male 7- to 12-week-old C57BL/6J mice (25–30 g; Jackson Laboratory, Bar Harbor, Maine), 7- to 15-week-old floxed *Bdnf* mice (25–32 g, BL6/sv129 background) (3), 2- to 3-month-old *Drd2* (D_2) green fluorescent protein (GFP) bacterial artificial chromosome transgenic mice (25–32 g, C57BL/6J background; Gene Expression Nervous System Atlas [GENSAT] Project; www.gensat.org) (23), and 4- to 6-month-old CD-1 retired breeders (35–45 g; Charles River Laboratories, Wilmington, Massachusetts) were used. Mice were fed ad libitum at 22–25°C on a 12-hour light/dark cycle. CD-1 mice were singly housed except during social defeats. All other mice were group housed before social defeats and singly housed after social defeats. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committees at Mount Sinai.

Stereotactic Surgeries for Pharmacologic and Optogenetic Approaches

Stereotactic surgeries were performed as described previously (8,24). For repeated optical activation of the VTA-NAc pathway during the CSDS paradigm, .5 μL of retrograde traveling adeno-associated virus (AAV2.5) vectors that express channelrhodopsin-2 (ChR2) fused with enhanced yellow fluorescent protein (EYFP) (AAV2.5-hsyn-ChR2-eYFP, purchased from University of Pennsylvania Vector Core, Philadelphia, Pennsylvania) was bilaterally infused into the NAc (anteroposterior +1.5; mediolateral ±1.5; dorsoventral −4.4 from bregma; 10° angle) at a rate of .1 μL/min. Three weeks later, optic fibers were bilaterally implanted into VTA (anteroposterior −3.2; mediolateral ±1.0; dorsoventral −4.6; 7° angle). If necessary, a bilateral 26-gauge guide cannula (4 mm length from the cannula base) was implanted bilaterally into NAc (anteroposterior +1.5; mediolateral +.75; dorsoventral −3.9; 0° angle) for drug infusions. Although these surgeries were targeted to the NAc medial shell, the manipulations also affected the NAc core because of the small size of this brain region in the mouse.

For optical activation of the VTA-NAc pathway in an acute defeat stress paradigm, as described previously (8), a double floxed (DIO) Cre-dependent AAV vector expressing ChR2 fused with EYFP (AAV-DIO-ChR2-EYFP, purchased from University of North Carolina Vector Core, Chapel Hill, North Carolina) was bilaterally infused into VTA. Two weeks later, a replication-defective version of the retrograde traveling pseudorabies virus expressing Cre (PRV-Cre, obtained from Jeffrey M. Friedman, Rockefeller University, New York, New York) was bilaterally infused into NAc. For localized *Bdnf* gene knockdown followed by repeated optogenetic activation of VTA-NAc pathway, AAV-Cre or AAV-GFP (purchased from University of North Carolina Vector Core) and AAV2.5-hsyn-ChR2-EYFP were infused into VTA and NAc of floxed *Bdnf* mice, respectively. Two weeks after the double surgery, optic fibers were bilaterally implanted into VTA, as described earlier.

Microinfusions

Approximately 15 minutes before daily defeat stress for 10 days, animals received bilateral intra-NAc infusions of SCH 23390 (D_1 receptor antagonist, 1 μg/.5 μL per side) (24), eticlopride (D_2 receptor antagonist, 1 μg/.5 μL per side) (24,25), or ANA-12 (TrkB inhibitor, 1 μg/.5 μL per side) (8), at doses known to be behaviorally active (8,24,25), or vehicle as a control (sterile saline or 50% dimethyl sulfoxide in artificial cerebrospinal fluid) at a continuous rate of .1 μL/min via a microinfusion pump (Harvard Apparatus, Holliston, Massachusetts). Injector needles remained in place for 5 minutes before being pulled out. Mice were allowed to sit undisturbed for ~5 minutes before the daily defeat stress. In the case of 1-day defeat stress, mice received a bilateral intra-NAc infusion of SCH 23390, eticlopride, or vehicle 1 hour before the social interaction test.

Social Defeat Stress Paradigm With Optogenetic Stimulation

Chronic stress and acute defeat stress were conducted as described previously. A social interaction test was performed 24 hours after the last defeat (3,4,6–8). Based on social interaction ratios (time in interaction zone with social target/time in interaction zone without social target × 100%), mice were designated as susceptible or resilient: susceptible ratio <100; resilient ratio ≥100. This measure of susceptibility versus resilience has been shown to correlate with other defeat-induced behavioral abnormalities (4). In vivo phasic stimulation of the VTA-NAc pathway was conducted (7) for 5 minutes immediately after or during the daily defeat stress in 10-day chronic defeat stress. Acutely defeated mice received the phasic stimulation during the social interaction test when CD-1 mice were presented for 2.5 minutes.

Ex Vivo Voltammetry

Fast scan cyclic voltammetry was used (26) to characterize presynaptic DA release and uptake and the ability of the DA terminal to respond to phasic stimulation patterns in the NAc shell. Animals were used for voltammetry experiments 18–22 hours after a social interaction test to identify susceptible mice after CSDS. To obtain baseline recordings from 400-μm-thick coronal brain sections containing the NAc shell, DA release was evoked by single-pulse stimulations (350 μA, 4 ms, monophasic) every 5 minutes. When a stable baseline was established (three collections within 10% variability), phasic

stimulation curves were run. We evaluated evoked DA release to single-pulse stimulations and multiple pulses (five stimulations at varying frequencies: 5–20 Hz).

Cocaine Conditioned Place Preference With Optogenetic Stimulation

An unbiased conditioned place preference (CPP) paradigm was used (24,27). Briefly, mice were placed in a three-chambered CPP box for 20 minutes to ensure no chamber bias. For the next 2 days of cocaine/light CPP, optic fibers were secured to the cannulae before saline or cocaine (10 mg/kg, intraperitoneal) injections. Mice were conditioned to saline/no light and cocaine/blue light (473 nm, 20-Hz frequency, bursts of 5 light-pulses, 40-ms pulse duration, every 10 seconds) for 30 minutes over 2 days. On the CPP test day, mice were allowed to freely explore all three chambers for 20 minutes. The CPP scores represent time spent in the paired chamber − time spent in the unpaired chamber.

Immunohistochemistry

Mice were anesthetized with a lethal dose of chloral hydrate and intracardially perfused with .1 mmol/L phosphate-buffered saline (PBS) and 4% (wt/vol) PBS-buffered paraformaldehyde 24 hours after the social interaction test. Post-fixed brains were incubated overnight in 30% sucrose at room temperature before being sliced on a microtome at 35 μm. Free-floating sections were washed with PBS and then blocked in 3% bovine serum albumin and .3% Triton X-100 (T8787; Sigma-Aldrich, St. Louis, Missouri) for 1 hour. For EYFP (ChR2)/tyrosine hydroxylase (TH) double labeling, 1:4000 of mouse anti-TH (T1299; Sigma-Aldrich) was used for overnight incubation with 1:1000 of chicken anti-GFP (GFP-1020; Aves Labs, Inc., Tigard, Oregon) at 4°C. The next day, 1:500 of donkey anti-mouse Cy3 (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pennsylvania) for anti-TH was used in PBS together with 1:500 of donkey anti-chicken Cy2 for anti-GFP. For GFP/extracellular signal-regulated kinase phosphorylation (pERK) double-labeling in D_2 GFP mice, brain sections were incubated in 1:1000 of chicken anti-GFP (Aves Labs, Inc.) and 1:1000 mouse anti-pERK (4370S; Cell Signaling Technology, Inc., Danvers, Massachusetts) in block solution overnight at 4°C. The next day, sections were rinsed in PBS and then incubated in 1:500 of donkey anti-chicken Cy2 (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pennsylvania) and 1:500 of donkey anti-mouse Cy3 in PBS for 1 hour and then rinsed in PBS. All sections were counterstained and mounted with antifade solution, including 4′,6-diamidino-2-phenylindole (VectaShield, H-1000; Vector Laboratories, Inc., Burlingame, California), then subsequently imaged on an LSM 710 confocal microscope (Carl Zeiss, Oberkochen, Germany). The GFP and pERK cell counting in the NAc was performed within a 200 μm × 200 μm square scale placed on the NAc shell or core. All immunopositive cells within the square scale were counted by an observer blind to experimental conditions.

Western Blotting

Bilateral 14-gauge NAc punch specimens were obtained from 1-mm coronal NAc sections from mice 24 hours after the social interaction test. Punch specimens were sonicated (Cole-Parmer, Vernon Hills, Illinois) in 30 μL of homogenization buffer containing 320 mmol/L sucrose, 5 nmol/L HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer, 1% sodium dodecyl sulfate (SDS), phosphatase inhibitor cocktails I and II (Sigma-Aldrich), and protease inhibitors (F. Hoffmann-La Roche Ltd., Basel, Switzerland). The concentration of protein was determined using the Bio-Rad DC Protein Assay (Bio-Rad, Hercules, California), and 25 μg of total protein was loaded onto a 18% gradient Tris-HCl polyacrylamide gel for electrophoresis fractionation (Bio-Rad). Samples were transferred onto a nitrocellulose membrane and blocked in Odyssey Blocking Buffer (LI-COR Biosciences, Lincoln, Nebraska) for 1 hour for LI-COR analysis. After blocking, the same membrane was incubated in 4°C overnight with either antibodies against BDNF (1:500, sc-546; Santa Cruz Biotechnology, Inc., Santa Cruz, California), detecting truncated BDNF, or β-tubulin (1:10,000, 2118; Cell Signaling Technology, Inc.) in Odyssey blocking buffer. After thorough washing with Tris-buffered saline and .1% Tween 20 (TBST), blots were incubated for 1 hour at room temperature with IRDye secondary antibodies (1:10,000; LI-COR Biosciences) in Odyssey blocking buffer. Blots were imaged with the Odyssey Infrared Imaging system (LI-COR Biosciences) and quantified by densitometry using ImageJ (National Institutes of Health, Bethesda, Maryland). The amount of protein blotted onto each lane was normalized to levels of tubulin.

Data Analysis

Data were analyzed with SigmaPlot 13.0 (Systat Software, Inc., San Jose, California) and Prism 6.0 (GraphPad Software, San Diego, California). Student *t* tests were used for the analysis of experiments with two experimental groups. One-way analysis of variance (ANOVA) was used for analysis of three or more groups, followed by Fisher's protected least significant difference post hoc tests, when appropriate. For social interaction data that were generated from the phasic stimulation experiment without pharmacologic infusions, two-way ANOVA was used followed by Fisher's protected least significant difference post hoc tests. For all analysis of voltammetry data, Demon Voltammetry and Analysis software was used (28). To evaluate DA kinetics, evoked levels of DA were modeled using Michaelis-Menten kinetics. Burst frequency response curves were subjected to a two-way repeated measures ANOVA with burst frequency as the within subjects factor and experimental group as the between subjects factor. p values $< .05$ were considered to be statistically significant. All data are expressed as mean ± SEM.

RESULTS

CSDS-Elicited Social Avoidance Is Mediated by BDNF Signaling, But Not by DA Signaling, in NAc

We first assessed the role of DA signaling in NAc using a 1-day defeat paradigm and an optogenetic method to activate the VTA-NAc pathway (Supplemental Figure S1A, B). Previous work showed that intra-NAc infusion of the TrkB inhibitor ANA-12 blocked social avoidance elicited by this acute stress

paradigm (8). We found that intra-NAc infusion of a D_1 receptor antagonist (SCH 23390), but not a D_2 receptor antagonist (eticlopride), blocked the ability of acute optogenetic stimulation of the VTA-NAc pathway to induce social avoidance in this 1-day stress procedure (Supplemental Figure S1C). These data demonstrate that both BDNF and DA signaling in NAc mediate the ability of acute optogenetic stimulation to induce social avoidance during acute social stress.

Although the 1-day defeat paradigm is useful to reveal prosusceptibility phenotypes (4,29), it has the major limitation of involving acute, not chronic, stress. Therefore, we used the standard CSDS (10–day) paradigm to determine the underlying mechanism in the VTA-NAc pathway responsible for CSDS-induced social avoidance. We infused bilaterally SCH 23390, eticlopride, or ANA-12 into NAc at a dose known to be behaviorally effective (8,24) 15 minutes before each daily defeat during the 10-day protocol (Figure 1A–C). As seen for the 1-day defeat paradigm, pretreatment with a TrkB inhibitor counteracted the social avoidance induced by CSDS (Figure 1D). However, neither D_1 nor D_2 receptor antagonist pretreatment blunted the social avoidance induced under these chronic stress conditions (Figure 1D).

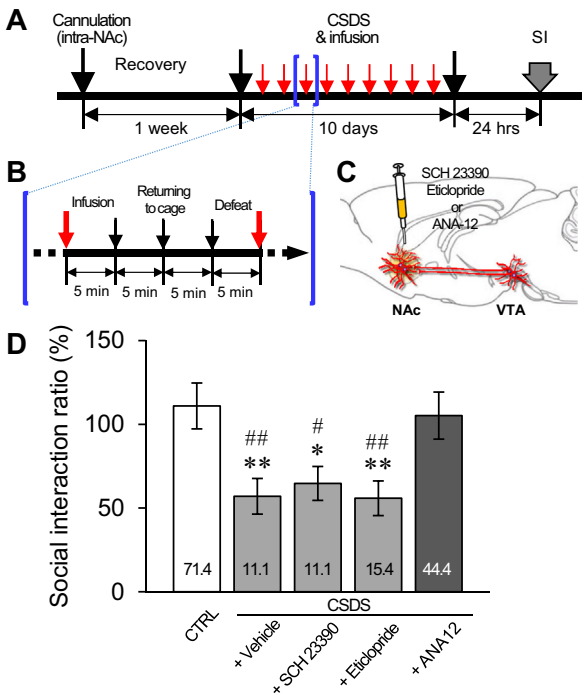

Figure 1. Role of brain-derived neurotrophic factor and dopamine signaling in nucleus accumbens (NAc) during chronic social stress. **(A)** Schematic diagrams depicting the experimental procedures for chronic social defeat stress (CSDS) and intra-NAc infusion of a D_1 receptor (SCH 23390, 1.0 μg/.5 μL/side), D_2 receptor (eticlopride, 1.0 μg/.5 μL/side), or tyrosine receptor kinase B (ANA-12, 1.0 μg/.5 μL/side) antagonist. **(B, C)** Drugs were infused into NAc 15 minutes before daily defeat events. Social interaction tests were performed 24 hours after the 10 daily defeats (gray arrows). **(D)** Intra-NAc infusion of a D_1 or D_2 receptor antagonist did not affect social avoidance induced by CSDS, but intra-NAc ANA-12 infusion blocked the social avoidance. The number in each bar indicates the percentage of resilient mice over total mice in each group. One-way analysis of variance ($F_{4,41} = 4.977$, $p < .01$, $n = 7$–13) with Fisher's protected least significant difference post hoc tests, $^{*}p < .05$, $^{**}p < .01$ compared with control group; $^{\#}p < .05$, $^{\#\#}p < .01$ compared with ANA-12 group. Bar graphs show mean ± SEM. CTRL, control; SI, social interaction; VTA, ventral tegmental area.

20-Hz Phasic Stimulation Worsens CSDS-Induced Social Avoidance

To determine the behavioral consequences of phasic stimulation of the VTA-NAc pathway during chronic stress compared with effects in the 1-day paradigm (7), we first injected retrograding AAV2.5-hsyn-ChR2-eYFP into NAc bilaterally and 3 weeks later implanted optic fibers in VTA (Figure 2A–C). At 4 weeks, ChR2 (EYFP-positive, green) was well expressed in VTA DA neurons (TH-positive, red) (Figure 2D). We then optically stimulated the VTA daily during CSDS using two stimulation protocols: stimulation during the defeat episodes (Figure 2E) versus immediately after each defeat (Figure 2F). The phasic stimulation during defeat had no additional effect on the social avoidance produced by CSDS. In contrast, the phasic stimulation post defeat exacerbated the effect of CSDS (Figure 2G). Phasic activation of the VTA-NAc pathway by itself (i.e., without defeat stress) had no effect on social interaction (Supplemental Figure S2).

Phasic Stimulation–Exacerbated Social Avoidance Is Prevented by BDNF Blockade

To investigate a role of BDNF in mediating the aggravated social avoidance induced by phasic stimulation of the VTA-NAc pathway during CSDS, we infused ANA-12 into NAc 15 minutes before each daily defeat for 10 days and optogenetically stimulated the pathway immediately after each defeat ("stimulation post defeat") (Figure 3A–C). Social interaction testing on day 6 revealed no changes in social behavior (Figure 3D). However, after 10 days of CSDS, phasic activation of the VTA-NAc pathway worsened social avoidance, and this effect was blocked by intra-NAc ANA-12 infusions (Figure 3E).

Consistent with these behavioral data, CSDS increased BDNF protein levels in NAc 24 hours after the social interaction test (Figure 3F). Post defeat phasic stimulation of the VTA-NAc pathway during CSDS ("CSDS Veh+ChR2") further increased BDNF levels compared with the nonstimulated defeated mice ("CSDS Veh+EYFP") (Figure 3F). ANA-12 treatment had no effect on BDNF protein levels (Figure 3F).

To complement the pharmacologic approach, we knocked down BDNF in the VTA of floxed *Bdnf* mice by local infusion of AAV-Cre. Control mice received intra-VTA injections of AAV-GFP. We found that although phasic stimulation of the VTA-NAc pathway during CSDS aggravated social avoidance in GFP control mice, this effect was lost in mice with a local VTA BDNF knockdown (Figure 3G–I). These data establish that BDNF expressed in VTA is required for the ability of repeated phasic stimulation of the VTA-NAc pathway to exacerbate social avoidance induced by chronic social stress.

Regulation of DA Release in NAc by CSDS

We employed ex vivo fast-scan cyclic voltammetry to investigate the effects of CSDS and optogenetic stimulation of the

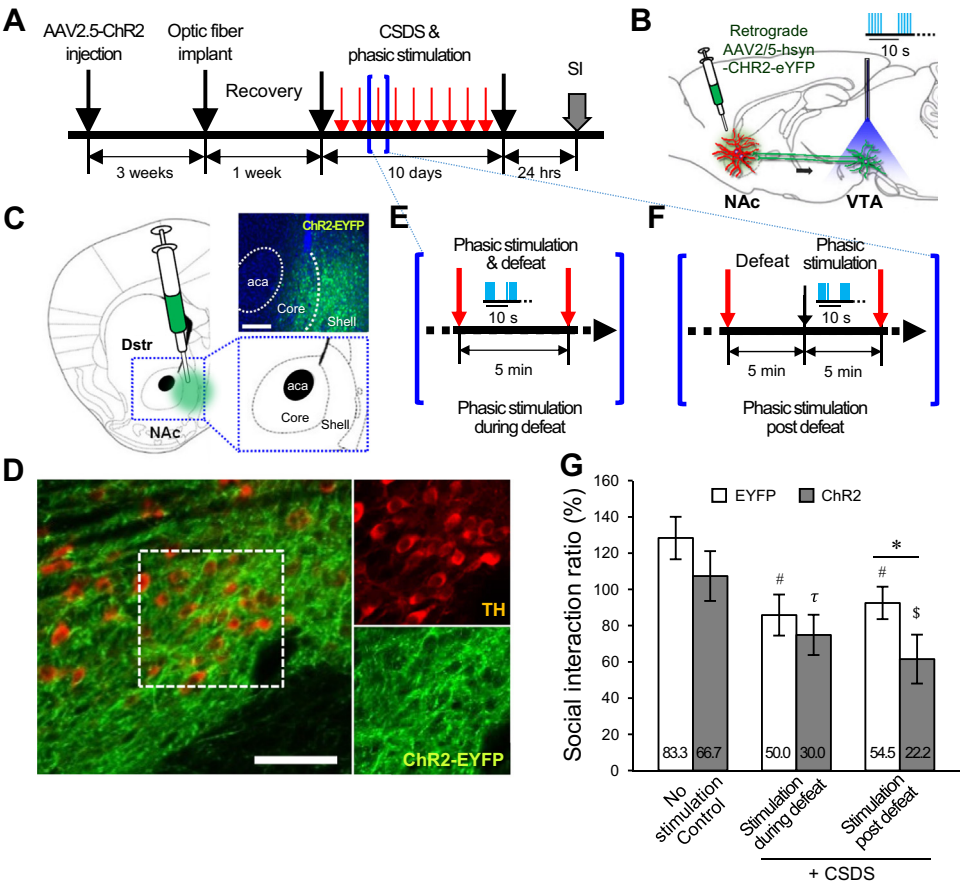

Figure 2. Effects of repeated optogenetic phasic stimulation of the ventral tegmental area (VTA)-nucleus accumbens (NAc) pathway during chronic social stress. **(A)** Schematic diagram depicting the experimental procedures for chronic social defeat stress (CSDS) and 20-Hz phasic VTA activation. **(B)** Schematic illustrating validation of NAc injection site. Scale bar = 100 μm. **(C)** Schematic of retrograde traveling adeno-associated virus (AAV2.5) vectors that express channelrhodopsin-2 (ChR2), fused with enhanced yellow fluorescent protein (EYFP) (AAV2.5-hsyn-ChR2-eYFP) infused into NAc and optic fiber implantation into VTA. **(D)** Representative confocal images showing localization of ChR2-EYFP (green) in tyrosine hydroxylase (TH)–positive cells (red) in VTA. Scale bar = 50 μm. **(E, F)** Two phasic stimulation protocols were used: 5-minute phasic stimulation was performed **(E)** during defeat episodes (phasic stimulation during defeat) or **(F)** immediately after defeat (phasic stimulation post defeat). **(G)** Phasic stimulation during defeat had no effect on social avoidance behavior induced by CSDS. However, phasic stimulation post defeat exacerbated the effect of CSDS. The number in each bar indicates the percentage of resilient mice over total mice in each group. Two-way analysis of variance (protocol effect [$F_{2,44} = 6.217$, $p < .01$], stimulation effect [$F_{1,44} = 4.574$, $p < .05$], protocol × stimulation effect [$F_{2,44} = .415$, $p = .663$], $n = 6$–11) with Fisher's protected least significant difference post hoc tests, $^{*}p < .05$ AAV-EYFP vs. AAV-ChR2 within each stimulation protocol; $^{\#}p < .05$ compared with control-EYFP group; $^{\tau}p < .1$, $^{\$}p < .05$ compared with control-ChR2 group. Bar graphs show mean ± SEM. aca, anterior part of anterior commissure; Dstr, dorsal striatum; SI, social interaction.

VTA-NAc pathway on DA release in NAc shell. We found that CSDS, which by itself produced social avoidance behavior (Figure 4A), did not alter electrically evoked DA release in NAc slices over broad frequencies (5–20 Hz) of stimulation (Figure 4B–D). Moreover, although phasic stimulation of the VTA-NAc pathway during CSDS worsened avoidance behavior (Figure 4A), it did not alter electrically evoked DA release (Figure 4B–D).

Cell Type–Specific Induction of pERK in NAc After CSDS

To assess the cell-type specificity of the effect of CSDS on BDNF-TrkB signaling in NAc, we measured levels of pERK, which is downstream of TrkB, after CSDS. We used D_2-GFP mice, which contain a bacterial artificial chromosome expressing GFP selectively in D_2-type medium spiny neurons (MSNs) (Figure 5A–C) (23,30). We found that 10 days of CSDS increased the number of pERK-positive/ D_2 receptor–negative cells in the NAc shell of susceptible mice, with no effect seen in resilient mice (Figure 5D, E). In contrast, CSDS had no effect on pERK immunoreactivity in D_2 receptor–positive cells (Figure 5F) or on total pERK-positive cell counts (one-way ANOVA [$F_{2,9} = 3.021$, p = not significant]). This effect was specific to the NAc shell, as no effect of CSDS was found for pERK immunoreactivity in either D_2 receptor–negative or D_2 receptor–positive core cells in susceptible or resilient mice (Supplemental Figure S3).

DISCUSSION

In the present study, we demonstrate that BDNF signaling, but not DA signaling, in the mesolimbic DA circuit is necessary for the susceptible phenotype produced by chronic social stress. The BDNF-TrkB blockade in NAc prevented CSDS-induced social avoidance behavior, whereas DA receptor antagonism did not. Repeated optogenetic phasic stimulation of the VTA-NAc circuit, which approximates enhanced burst firing of the pathway that occurs uniquely in susceptible mice (4,6,7), increased BDNF levels in NAc and aggravated CSDS-induced social avoidance behavior. This exaggerated susceptible phenotype too was prevented by BDNF-TrkB blockade in NAc and by localized BDNF knockdown in VTA. These data agree with previous studies showing that NAc BDNF, transported from VTA, is critical for the susceptible phenotype after

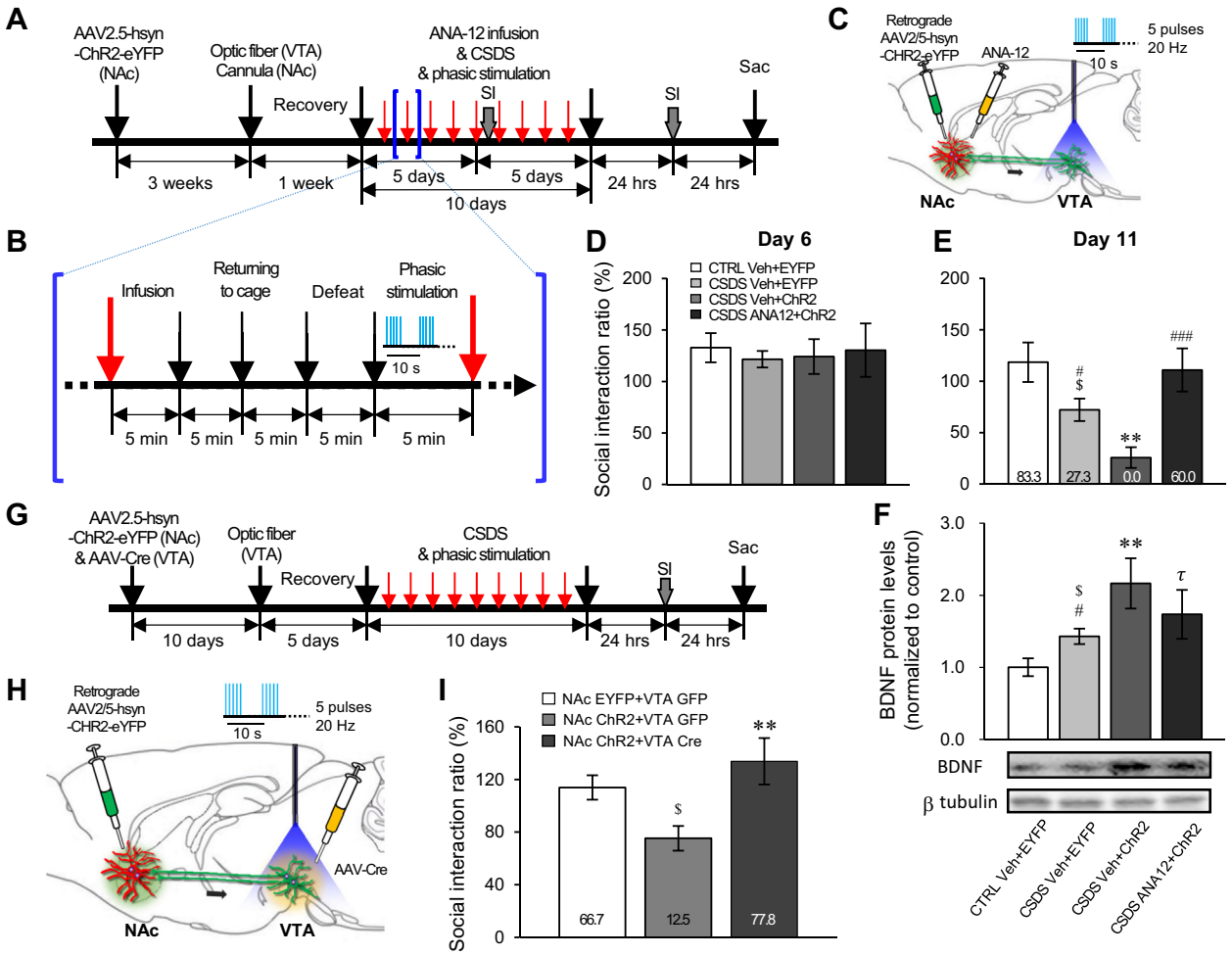

Figure 3. Role of brain-derived neurotrophic factor (BDNF) signaling in nucleus accumbens (NAc) during repeated phasic optogenetic stimulation of the ventral tegmental area (VTA)-NAc pathway and chronic social stress. **(A, B)** Schematic diagrams depicting the experimental procedures for intra-NAc ANA-12 infusions and 20-Hz optical activation during chronic social defeat stress (CSDS). Social interaction tests were performed 24 hours after the 5 daily defeats and again 24 hours after 10 daily defeats (gray arrows). **(C)** Schematic of retrograde traveling adeno-associated virus (AAV2.5) vectors that express channelrhodopsin-2 (ChR2), fused with enhanced yellow fluorescent protein (EYFP) (AAV2.5-hsyn-ChR2-eYFP) infused into NAc, intra-NAc ANA-12 infusions, and optic fiber implantation into VTA. **(D)** Defeat stress for 5 days plus phasic activation had no effect on social avoidance (one-way analysis of variance [$F_{3,31}$ = .0813, p = not significant, n = 6–11]). **(E)** Defeat stress for 10 days reduced social interaction, and this impaired social interaction was exacerbated by repeated phasic activation of the VTA-NAc pathway. Additional post hoc analyses with unpaired t tests showed that the CSDS Vehicle+EYFP group had lower social interaction time than the control Vehicle+EYFP group (t_{15} = 2.277, $^{\$}p < .05$, n = 6, 11). ANA-12 infusion into NAc totally reversed the effects of optogenetic stimulation on social avoidance ($F_{3,31}$ = 6.360, $p < .01$, n = 6–11). The number in each bar indicates the percentage of resilient mice over total mice in each group. **(F)** Repeated phasic stimulation of the VTA-NAc pathway during CSDS increased BDNF protein levels in NAc ($F_{3,23}$ = 3.485, $p < .05$, n = 5–9). Additional post hoc analyses with unpaired t tests showed that BDNF protein levels in the CSDS Vehicle+EYFP group are higher than the levels in the control Vehicle+EYFP group (t_{12}= 2.508, $^{\$}p < .05$, n = 5, 9). One-way analysis of variance with Fisher's protected least significant difference post hoc tests, $^{\tau}p < .1$, $^{**}p < .01$ compared with control Vehicle+EYFP group; $^{\#}p < .05$, $^{\#\#}p < .001$ compared with CSDS Vehicle+ChR2 group. **(G)** Schematic diagrams depicting the experimental procedures for localized genetic depletion of VTA *Bdnf* and 20-Hz optogenetic activation of VTA during CSDS. **(H)** Retrograde AAV2.5-hsyn-ChR2-eYFP was infused into NAc for optogenetic activation of the VTA-NAc pathway. AAV-Cre was infused into VTA of floxed *Bdnf* mice for localized knockdown of BDNF expression in VTA. **(I)** Knockdown of BDNF in VTA reversed the detrimental effect of repeated optogenetic VTA stimulation on social interaction ($F_{2,23}$ = 4.823, $p < .05$, n = 8–9). Additional post hoc analyses with unpaired t tests showed that phasic stimulation of the VTA-NAc pathway reduced social interaction (NAc EYFP+VTA green fluorescent protein [GFP] group vs. NAc ChR2+VTA GFP group [t_{15}= 2.700, $^{\$}p < .05$, n = 8, 9]). Bar graphs show mean ± SEM. CTRL, control; Sac, sacrifice; SI, social interaction.

CSDS (3,4,8) and establish BDNF signaling by VTA DA neurons as an abnormal, pathologic mechanism that arises during a period of chronic stress.

In our study, CSDS-induced social avoidance was blocked by neither D_1 nor D_2 receptor antagonism, even though repeated, severe stress exposure facilitates DA release in the NAc shell (31–33). Our observations suggest that increased DA transmission in NAc during severe stress is not associated with depressive-like phenotypes. Moreover, we show that CSDS has no effect on electrically evoked DA release in NAc ex vivo compared with stress-naïve animals. Previous clinical and animal studies showed a negative correlation

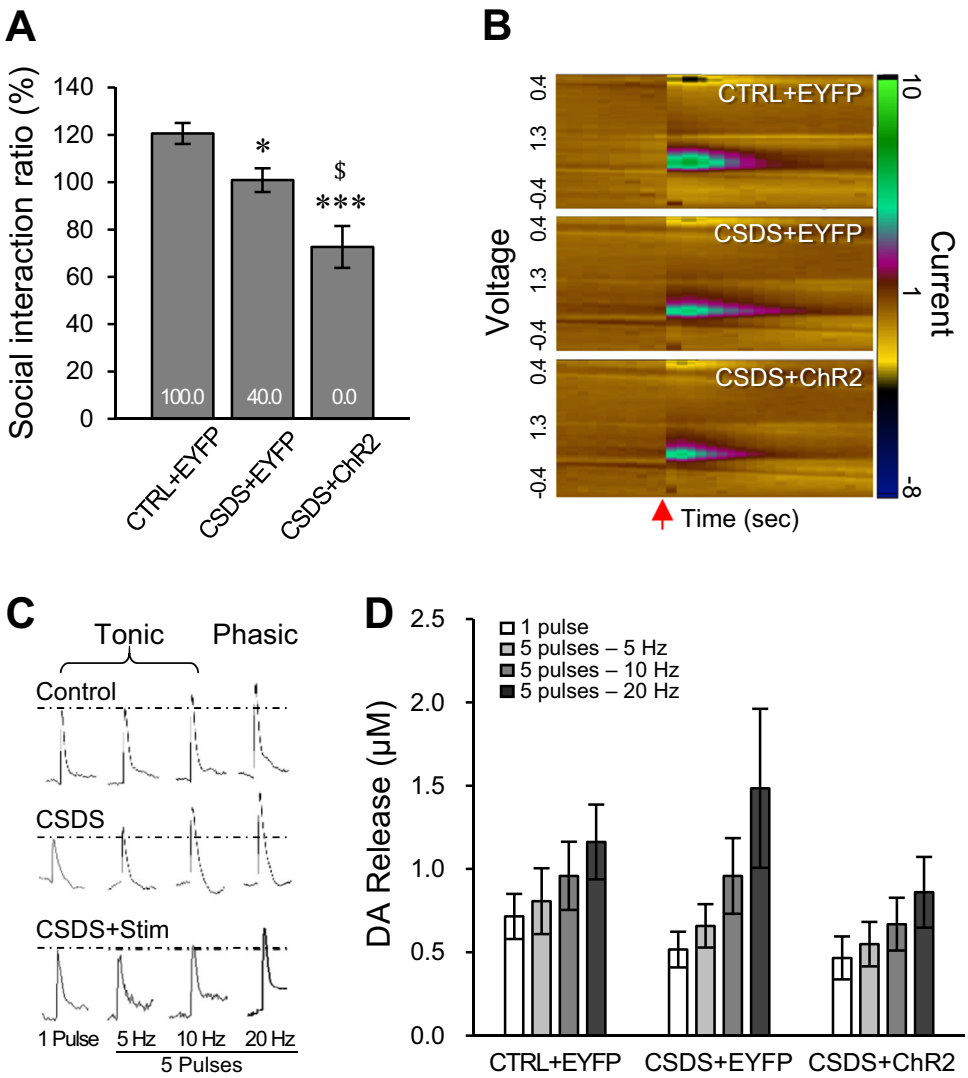

Figure 4. Kinetics of dopamine (DA) release in nucleus accumbens (NAc) after optogenetic activation of ventral tegmental area (VTA) and chronic social stress using ex vivo fast scan cyclic voltammetry. **(A)** Repeated chronic social defeat stress (CSDS) induced social avoidance, and this effect was exacerbated by repeated phasic optogenetic stimulation of the VTA-NAc pathway. One-way analysis of variance ($F_{2,15}$ = 10.481, $p < .001$, n = 5–7) with Fisher's protected least significant difference post hoc tests, $^{*}p < .05$, $^{***}p < .001$ compared with the control plus enhanced yellow fluorescent protein (CTRL+EYFP) group. Additional post hoc analyses with unpaired t tests showed that repeated phasic stimulation of the VTA-NAc pathway aggravated the detrimental effect of CSDS on social interaction (CSDS+EYFP group vs. CSDS plus channelrhodopsin-2 [CSDS+ChR2] [t_{11}= 2.651, $^{\$}p < .05$, n = 6, 7]). **(B)** Color plots showing evoked DA release from CTRL+EYFP, CSDS+EYFP, and CSDS+ChR2 groups. **(C)** Evoked DA release to single pulse and five pulses across frequencies (range, 5–20) highlighting the frequency response in the magnitude of DA in NAc shell. **(D)** Group data demonstrating no effect of CSDS ± repeated phasic activation of the VTA-NAc pathway on DA release. Two-way analysis of variance (group effect [$F_{2,60}$ = 2.522, p = .0888], stimulation effect [$F_{3,60}$ = 4.935, $p < .01$], group × stimulation effect [$F_{6,60}$ = .445, p = .846], n = 5–7). Bar graphs show mean ± SEM. The number in each bar indicates the percentage of resilient mice over total mice in each group. No differences were seen between susceptible and resilient mice (not shown).

between concentrations of DA metabolites and depressive symptoms, but a positive correlation between antidepressant effects and DA transmission in NAc shell (see introduction). However, in contrast to the lack of involvement of DA signaling in NAc after CSDS, D_1, but not D_2, receptor antagonism in NAc—similar to acute BDNF-TrkB antagonism (8)—blocked the ability of acute optogenetic activation of the VTA-NAc pathway to worsen the effects of acute stress. These data suggest that very different mechanisms are at play during responses to initial stress compared with more pathologic changes that occur with chronic stress. Repeated, excessive stress may promote greater release of BDNF, but not of DA, from VTA nerve terminals, resulting in depressive-like pathologies (34).

We provide evidence that D_1 receptor MSNs are the site of action of BDNF in NAc after CSDS. We show that pERK levels are increased solely in D_2 receptor–negative cells in the NAc shell of susceptible mice, with no change in resilient mice. Prior work established that GFP-negative cells in D_2-GFP mice provide a highly reliable measure of D_1-type MSNs (35,36). This finding suggests that BDNF signaling in D_1 receptor MSNs contributes to the susceptible phenotype after CSDS. Enhanced ERK phosphorylation has been associated with a reduction in neuronal activity of D_1 receptor MSNs (27), and reducing neuronal activity of D_1 receptor MSNs renders resilient mice more susceptible (37). Moreover, excitatory synaptic input to D_1 receptor MSNs is reduced in susceptible mice after CSDS (37) and in mice subjected to repeated restraint stress (38). Together, these results support a scheme wherein increased BDNF signaling in NAc contributes to CSDS-induced behavioral susceptibility by inhibiting the activity of D_1 receptor MSNs. An important caveat is that ERK is downstream of several signaling pathways in addition to BDNF; therefore, further work is needed to directly test this and alternative hypotheses.

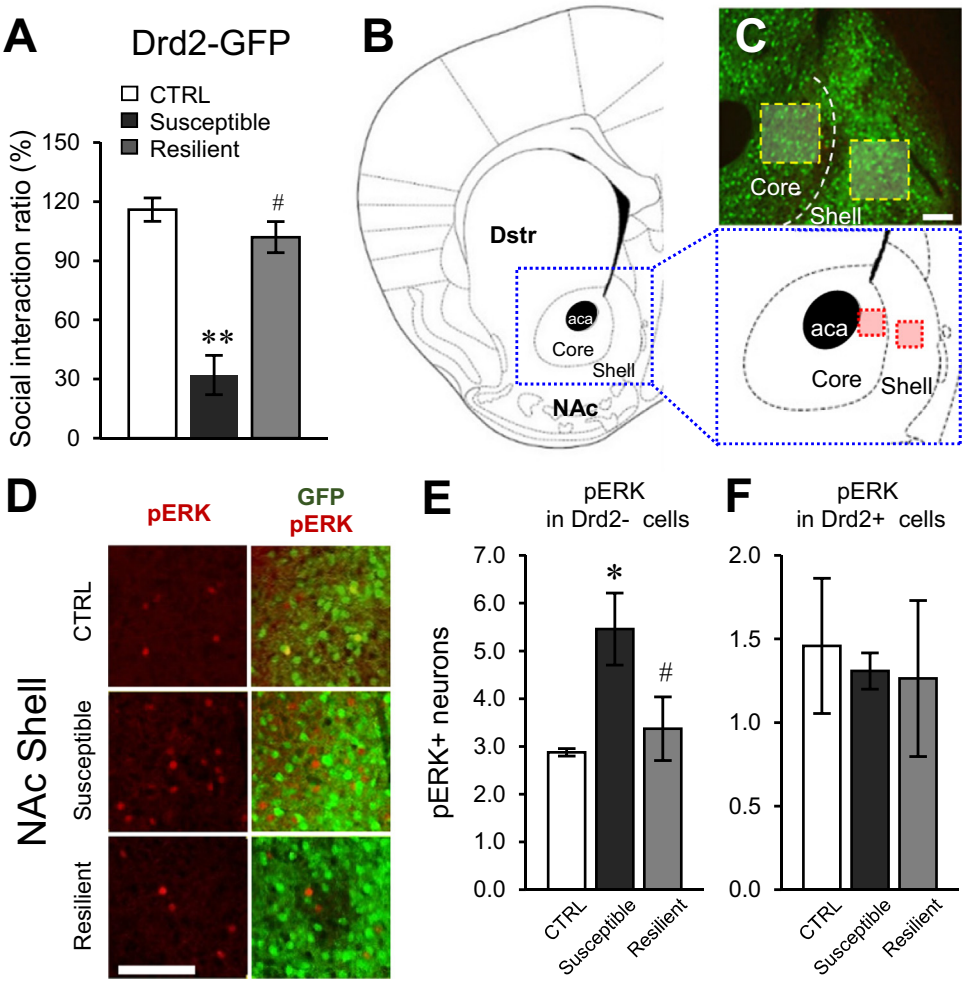

Figure 5. Cell type–specific extracellular signal-regulated kinase phosphorylation (pERK) by chronic social stress. **(A)** Chronic social defeat stress induces social avoidance in susceptible, but not resilient, *Drd2* (D_2) mice expressing green fluorescent protein (GFP) (one-way analysis of variance [$F_{2,9}$ = 30.665, $p < .001$, n = 4]). **(B)** Schematic of coronal sections of nucleus accumbens (NAc), with insets showing representative counting zone (200 μm × 200 μm) of GFP immune-positive D_2 medium spiny neurons. **(C)** Confocal images showing D_2 GFP immune-positive medium spiny neurons in NAc core and shell with insets showing representative counting zones. Scale bar = 100 μm. **(D)** Representative confocal images showing pERK-positive (red) cells and D_2-GFP-positive neurons (green) in NAc shell. Scale bar = 100 μm. **(E)** Number of pERK-positive/D_2-negative cells in NAc shell of susceptible mice was higher than that seen in resilient and stress-naïve mice ($F_{2,9}$ = 5.517, $p <$ .05). The number of pERK-positive cells is comparable to the number reported previously for this brain region (41). **(F)** However, there was no difference in number of pERK-positive/D_2-positive cells in NAc shell ($F_{2,9}$ = .0796, p = not significant). One-way analysis of variance with Fisher's protected least significant difference post hoc tests, $^*p < .05$, and $^{**}p < .01$ compared with control group; $^{\#}p < .05$ compared with susceptible group. Bar graphs show mean ± SEM. aca, anterior part of anterior commissure; CTRL, control; Dstr, dorsal striatum.

Other investigators have also implicated BDNF signaling in NAc in drug reward, particularly for cocaine (27,39). We observed that blockade of BDNF signaling in NAc inhibits cocaine reward (Supplemental Figure S4) in addition to CSDS-induced social avoidance and that both behaviors are enhanced by optogenetic activation of the VTA-NAc pathway. However, we demonstrated previously that enhancement of cocaine reward by BDNF in NAc is mediated by D_2 receptor MSNs (27). This is in contrast to the present study, in which we provide evidence that NAc BDNF-TrkB enhancement of stress susceptibility is mediated by D_1 receptor MSNs, establishing very different mechanisms for the ability of BDNF acting in NAc to promote drug reward versus behavioral susceptibility to chronic stress.

In conclusion, the present study demonstrates a required role of mesolimbic BDNF signaling, rather than DA signaling, in NAc in mediating social avoidance induced by CSDS. Our data suggest that NAc BDNF, which originates from VTA (3), mediates social avoidance through activation of TrkB on D_1 receptor MSNs, as evidenced by exclusive induction of ERK phosphorylation in D_1 receptor MSNs of susceptible mice. Our findings in chronically stressed animals and animals submitted to chronic optogenetic stimulation demonstrate clear differences from findings with acute stress and acute stimulation paradigms, as D_1 receptor DA function in NAc is involved in acute responses, but not chronic responses. Thus, our findings address the paradox of why increased firing of VTA DA neurons, which might otherwise be expected to be associated with increased reward and decreased depression-like behavior, is causally linked to susceptibility to CSDS. The sustained activation of these neurons, in the context of chronic stress, promotes increased release of BDNF, which produces pathologic effects within the mesolimbic DA circuit. This prodepressant role of mesolimbic BDNF signaling is in direct contrast to the antidepressant-like actions of BDNF in the hippocampus, which emphasizes the circuit-specific nature of molecular mechanisms involved in brain disease (40).

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