BDNF-TrkB controls cocaine-induced dendritic spines in rodent nucleus accumbens dissociated from increases in addictive behaviors

Ethan M. Anderson[a], Anne Marie Wissman[a], Joyce Chemplanikal[a], Nicole Buzin[a], Daniel Guzman[a], Erin B. Larson[a], Rachael L. Neve[b], Eric J. Nestler[c], Christopher W. Cowan[d], and David W. Self[a,1]

[a]Department of Psychiatry, The Seay Center for Basic and Applied Research in Psychiatric Illness, UT Southwestern Medical Center, Dallas, TX 75390; [b]Viral Gene Transfer Core, Massachusetts Institute of Technology, Cambridge, MA 02139; [c]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029; and [d]Department of Neuroscience, Medical University of South Carolina, Charleston, SC 29425

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Chronic cocaine use is associated with prominent morphological changes in nucleus accumbens shell (NACsh) neurons, including increases in dendritic spine density along with enhanced motivation for cocaine, but a functional relationship between these morphological and behavioral phenomena has not been shown. Here we show that brain-derived neurotrophic factor (BDNF) signaling through tyrosine kinase B (TrkB) receptors in NACsh neurons is necessary for cocaine-induced dendritic spine formation by using either localized TrkB knockout or viral-mediated expression of a dominant negative, kinase-dead TrkB mutant. Interestingly, augmenting wild-type TrkB expression after chronic cocaine self-administration reverses the sustained increase in dendritic spine density, an effect mediated by TrkB signaling pathways that converge on extracellular regulated kinase. Loss of TrkB function after cocaine self-administration, however, leaves spine density intact but markedly enhances the motivation for cocaine, an effect mediated by specific loss of TrkB signaling through phospholipase Cgamma1 (PLCγ1). Conversely, overexpression of PLCγ1 both reduces the motivation for cocaine and reverses dendritic spine density, suggesting a potential target for the treatment of addiction in chronic users. Together, these findings indicate that BDNF-TrkB signaling both mediates and reverses cocaine-induced increases in dendritic spine density in NACsh neurons, and these morphological changes are entirely dissociable from changes in addictive behavior.

accumbens shell | cocaine addiction | BDNF-TrkB | PLC | dendritic spines

Cocaine addiction is a chronic, debilitating disorder with strong negative effects on society and a lack of effective treatments (1). One potential target for treatment is brain-derived neurotrophic factor (BDNF) and its tyrosine receptor kinase B (TrkB) (2–5). BDNF is critical for normal brain development in early life stages, but also plays a role in neuronal maintenance and neuroplasticity in the mature brain (6). We previously found that limited daily access to cocaine self-administration (CSA) induces transient increases in BDNF and TrkB levels in the nucleus accumbens shell (NACsh) (2, 3), and that inducible and localized deletion of either BDNF or TrkB in NACsh neurons reduces CSA behavior (2, 3). Others have shown that overexpression of BDNF in the NAC increases cocaine sensitization and conditioned place preference (4). Since deleting BDNF-TrkB signaling in NACsh neurons can reduce addictive behavior, drugs that block BDNF-TrkB signaling could be an effective treatment for cocaine addiction (7).

In addition to altering BDNF-TrkB signaling, cocaine also induces dendritic spine formation in NAC neurons (8). Dendritic spine formation is functionally linked to activity-dependent BDNF-TrkB signaling in hippocampal pyramidal neurons (9), but whether local BDNF-TrkB signaling is involved in cocaine-induced spine formation in medium spiny NACsh neurons is unknown. Thus, we tested whether BDNF-TrkB signaling is necessary for cocaine-induced dendritic spine formation in NACsh neurons. Using transient viral-mediated expression of a dominant negative TrkB mutant, we also tested whether BDNF-TrkB signaling is important for maintaining the stability of cocaine-induced spines once formed and, surprisingly, found that BDNF-TrkB signaling plays opposing roles in the induction and maintenance of cocaine-induced dendritic spines.

BDNF binds to cell surface TrkB receptors to activate neurons by via numerous intracellular pathways (6). BDNF activation of TrkB autophosphorylates several tyrosines, including TYR515 and TYR816, that produce differential effects on neurite growth, survival, and neuroplasticity (6). Autophosphorylation of TYR816 recruits and phosphorylates PLCγ; this leads to downstream regulation of protein kinase C and calcium signaling (10). Our prior work found that CSA increases PLCγ phosphorylation (pPLC) in NACsh in a BDNF-dependent manner (3). Autophosphorylation of another TrkB site, TYR515, recruits a docking complex containing Src homology 2 domain-containing protein (SHC), culminating in Ras-dependent extracellular regulated kinase (ERK) phosphorylation (pERK) and activation. ERK has a prominent role in mediating BDNF-induced dendritic spine formation in hippocampal neurons (9) and could therefore play a role in spine formation in NAcSh neurons after chronic CSA. Thus, we compared the necessary and sufficient roles of these distinct TrkB signaling pathways

Significance

Chronic cocaine use leads to both increased motivation for the drug and long-lasting morphological changes in the form of increased dendritic spine density in medium spiny neurons of the nucleus accumbens. Here we show that the cocaine-induced morphological changes are mediated by local brain-derived neurotrophic factor (BDNF)-tyrosine kinase B (TrkB) signaling, similar to activity-dependent dendritic spine increases in other brain regions. However, the same BDNF-TrkB signaling pathway can reverse these spine increases after their formation, suggesting BDNF-TrkB mediates a form of "cataplasticity" that resets accumbens neurons to their preaddiction morphological state. Importantly, however, the changes in spine density are dissociated from BDNF-TrkB effects on motivation for cocaine, demonstrating that the increased dendritic spine density is likely not a causal factor in maintaining cocaine addiction.

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[1]To whom correspondence should be addressed. Email: david.self@utsouthwestern.edu.

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on modulation of cocaine-induced dendritic spines, along with the modulation of CSA behavior. Ultimately, our findings indicate that postcocaine activation of PLCγ signaling reduces the motivation for cocaine completely independent of effects on morphological changes in NACsh neurons.

Results

Given the role of BDNF-TrkB activity in dendritic spine formation in other brain regions (e.g., hippocampus) (9), we hypothesized that TrkB would have a critical role in the induction of cocaine-induce dendritic spines in medium spiny neurons of the NACsh. We used two methods to attenuate TrkB function in NACsh neurons. First, a herpes simplex viral vector (HSV)-encoding Cre recombinase with bicistronic GFP expression was infused into the NAC of floxed TrkB mice to delete the TrkB gene selectively from NAC neurons. Second, we infused an HSV vector expressing a kinase-dead (KD) dominant negative TrkB mutant (HSV-TrkB-KD) in the NAC. Fig. 1*A* shows that ectopic expression of either Cre or TrkB-KD aligned with GFP labeling in NACsh neurons. HSV-Cre expression in the NAC produced no evidence for retrograde infection in areas projecting to the NACsh (Fig. S1). Neither HSV-Cre nor HSV-TrkB-KD expression altered baseline dendritic spine density in distal dendrites of medium spiny neurons of the NACsh in saline-treated animals compared with control infection with HSV-GFP alone (Fig. 1 *B* and *C*). After a binge-like cocaine dosing regimen previously shown to induce an increase in spine density during HSV peak expression times (5 × 20 mg/kg i.p. injections over the course of 3 d) (11), total spine density appeared to increase in HSV-GFP–infected controls, but not in neurons expressing Cre or TrkB-KD (effect of vector, $F_{2,18} = 5.286$; $P = 0.0156$), although a lack of significant interaction precludes pairwise comparisons (Fig. 1 *B* and *C*). However, further analysis of spine morphology indicates that binge cocaine exposure induced primarily thin (Fig. 1*D*), but not mushroom-shaped (Fig. 1*E*) or stubby, spines (Fig. 1*F*), consistent with the profile for immature early spine induction immediately after similar cocaine treatment regimens (11). The thin spines were prevented from forming by either localized TrkB knockout or dominant negative TrkB mutant expression in NACsh neurons [Fig. 1*D*; effect of vector, $F_{2,18} = 6.616$ ($P = 0.007$); effect of drug, $F_{1,18} = 8.051$ ($P = 0.0109$); interaction, $F_{2,18} = 4.964$ ($P = 0.0192$)]. These results demonstrate that local BDNF-TrkB signaling in NACsh neurons is necessary for the increase in spine density induced by cocaine. Together with our previous work showing that CSA induces BDNF expression, release, and TrkB activation in NACsh, and that BDNF or TrkB knockout in NAC neurons decreases CSA behavior (2, 3), these findings indicate BDNF-TrkB signaling during initial cocaine exposure is necessary for both the induction of morphological plasticity and the maintenance of cocaine reinforcement.

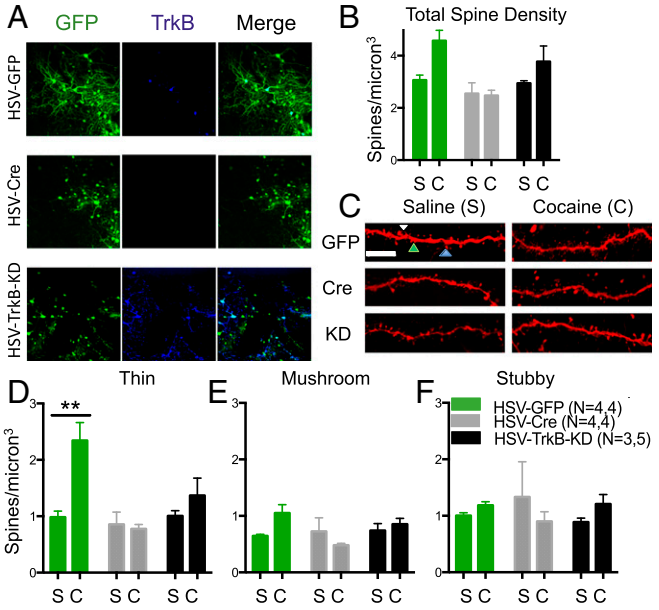

Fig. 1. Cocaine induction of spines is blocked by TrkB knockdown. (*A*) Localized expression of GFP (green) and TrkB (blue) after intra-NACsh infusions of HSV-GFP, HSV-Cre, or HSV-TrkB-KD (with bicistronic GFP) in floxed TrkB mice. (Magnification, 10×.) (*B*) Total spine density of saline- and cocaine-treated (5 × 20 mg/kg, i.p.) mice during HSV expression. (*C*) Representative images of saline- and cocaine-treated dendrites expressing GFP only, Cre recombinase, or TrkB-KD. Representative spines are labeled white (thin), blue (mushroom), and green (stubby) to illustrate their categorization. (Scale bar, 10 μm.) (*D*) Thin, (*E*) mushroom, and (*F*) stubby spine density after binge cocaine-dosing paradigm. Data are expressed as mean ± SEM. $^{**}P < 0.01$ compared with saline.

Cocaine-induced spines can remain significantly increased for a month after CSA (12, 13), and we hypothesized that knocking down TrkB function after chronic CSA would reverse these spine increases similar to blocking their induction. We compared the effects of knocking down TrkB signaling with HSV-TrkB-KD to the effects of increasing TrkB function by overexpressing wild-type TrkB (HSV-TrkB-WT). We also investigated the role of specific TrkB signaling pathways TrkB-SHC or TrkB-PLCγ. This was accomplished by constructing HSV vectors expressing two additional TrkB mutants: HSV-TrkB-515/SHC contains a phosphorylation-resistant tyrosine to phenylalanine mutation at TYR515 that prevents TrkB coupling with SHC complexes to activate ERK, but preserves TrkB-PLCγ signaling (10). Conversely, HSV-TrkB-816/PLC has a phosphorylation-resistant mutation of TYR816 that prevents TrkB-PLCγ signaling while leaving TrkB-SHC activation of pERK intact.

In heterologous cells in culture (HEK293), all four vectors increased TrkB protein expression, but only TrkB-WT enhanced BDNF-TrkB-mediated phosphorylation of both PLCγ and ERK compared with no vector or HSV-GFP–infected controls. In contrast, HSV-TrkB-KD had no detectable increases in BDNF-TrkB-WT-induced pPLCγ and pERK. Cells treated with HSV-TrkB-816/PLC lacked BDNF-stimulated PLCγ phosphorylation, but showed marked BDNF-induced pERK, whereas cells treated with HSV-TrkB-515/SHC had very low BDNF-induced pERK, but showed increased pPLCγ similar to HSV-TrkB-WT (Fig. 2*A* and Fig. S2 *A–D*).

In HEK293 cells that lack endogenous TrkB expression, the TrkB signaling mutants form homodimers that generally act to enhance TrkB function through their remaining intact signaling pathway (as shown in Fig. 2*A*). However, when expressed in combination with WT TrkB, they can mediate pathway-selective, dominant negative effects on their mutated signaling pathway via formation of heterodimers with WT TrkB (14), similar to TrkB-KD. To demonstrate the specificity of these signaling phenomena, Fig. 2*B* shows that HSV-TrkB-816/PLC attenuated WT TrkB-mediated pPLCγ while maintaining pERK similar to TrkB-WT alone. The pathway-specific effects of HSV-TrkB-515/SHC are less prominent, as BDNF-induced pERK is reduced but not completely blocked, while pPLCγ is retained (Fig. 2*B*). One possibility is that intact PLCγ signaling can indirectly lead to pERK through downstream Ca^{2+} signaling cascades (10) (and see following). The pPLCγ and pERK response to intra-NACsh BDNF infusions in vivo showed similar dominant negative profiles in animals expressing these HSV-TrkB vectors (Fig. S2 *E–J*). These complementary gain and loss of signaling interactions with WT TrkB are summarized in Fig. 2*C* for clarity. Importantly, the role of gain versus loss of pathway-specific TrkB signaling in mediating

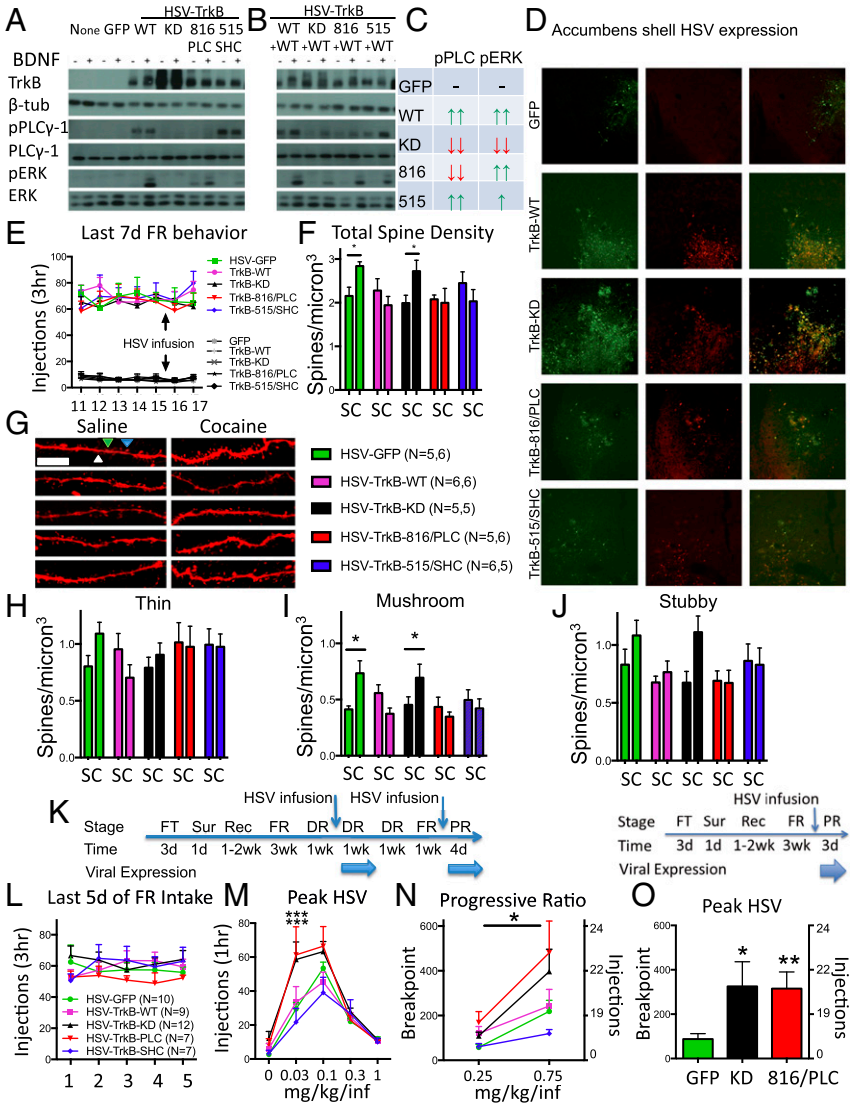

Fig. 2. BDNF-TrkB signaling reverses dendritic spines induced by chronic CSA and is dissociated from increased CSA behaviors caused by the selective loss of BDNF-TrkB-PLC activity in NACsh neurons. (*A*) Protein levels of TrkB, β-tubulin, pPLCγ, PLCγ, pERK, and ERK either with or without the addition of BDNF. Results from HEK293 cells infected with no vector, HSV-GFP, HSV-TrkB-WT, HSV-TrkB-KD, HSV-TrkB-816/PLC, or HSV-TrkB-515/SHC alone. (*B*) Coinfection with HSV-TrkB-WT reveals pathway-specific dominant negative profiles of TrkB signaling mutants. (*C*) Summary of TrkB-WT and dominant negative TrkBs HSV on pPLC and pERK. (*D*) Localized expression of GFP (green) and TrkB (red) 3 d after intra-NACsh infusions of HSV-GFP and HSV-TrkB vectors. (Magnification, 10×.) (*E*) Cocaine and saline SA rates before and after infusions of HSV vectors. (*F*) Quantification of total spine density in saline and CSA expressing TrkB viral vectors. (*G*) Representative images of dendritic segments expressing HSV vectors from saline and CSA animals. Representative spines are labeled white (thin), blue (mushroom), and green (stubby) to illustrate their categorization. (Scale bar, 10 μm.) (*H*) Quantification of thin, (*I*) mushroom, and (*J*) stubby spine density after SA. (*K*, *Left*) Experimental time course depicting operant training with food pellets (FT), surgery (Sur), recovery (Rec), and fixed ratio (FR) CSA training, followed by dose–response (DR) before, during, and after HSV infusion. A second HSV infusion is given before CSA testing on a PR reinforcement schedule. (*K*, *Right*) A modified experimental time course depicting 1 HSV infusion before PR. (*L*) Average CSA (FR5) in study groups before (*M*) dose–response testing during peak HSV-mediated expression. (*N*) CSA on a PR schedule during HSV expression after a second HSV infusion; the asterisk above a line represents a significant main effect of vector after a mixed factorial ANOVA. (*O*) CSA (0.75 mg/kg/injection) on a PR schedule during HSV expression after a single HSV infusion. Data are expressed as mean ± SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ compared with HSV-GFP. C, cocaine; S, saline.

morphological and behavioral effects can only be revealed when comparable effects are produced by WT (gain) or KD (loss) TrkB.

After 3 wk of CSA in daily 3-h sessions, rats were given intra-NACsh infusions of HSV vector and allowed access to CSA for 2 additional days (Fig. 2*E*). Brain tissue was collected 1 d after the final SA session for subsequent analysis of spine density. No viral vector significantly altered CSA baselines during the final 2 d of SA training when rats had relatively unrestricted access to cocaine injections (1 lever press for each injection). Moreover, blockade of endogenous TrkB signaling with HSV-TrkB-KD failed to affect the increase in total spine density induced by chronic CSA compared with GFP-only–expressing neurons (Fig. 2 *F* and *G*), in marked contrast to its ability to prevent the initial induction of spine formation (Fig. 1). Instead, surprisingly, overexpression of WT TrkB completely reversed the increase in total spine density produced by chronic CSA (drug × vector interaction, $F_{4,46} = 2.726$; $P = 0.041$), and spine density levels were similar to those seen in GFP-expressing neurons from saline SA animals. In contrast to early spine induction shown in Fig. 1, increases in spine density after chronic CSA were represented by increases in mushroom-shaped spines, but not thin or stubby spines (Fig. 2 *H–J*), consistent with maturation and stabilization of the newly formed thin dendritic spines with prolonged cocaine use (15). However, the increase in mushroom-shaped spines at the end of CSA was reversed by TrkB-WT, TrkB-816/PLC, and TrkB-515/SHC overexpression (Fig. 2*I*; drug × vector interaction, $F_{4,46} = 4.078$; $P = 0.0065$). Moreover, a similar TrkB-WT reversal of cocaine-induced mushroom spines was seen after 15 d of experimenter-delivered i.p. cocaine injections in mice (Fig. S3). Thus, BDNF-TrkB activity has opposing roles on the induction versus stability of cocaine-induced dendritic spines in NACsh neurons, being both necessary for spine formation and sufficient for their later reversal, regardless of the mode of cocaine administration.

Since WT, but not KD, TrkB produces a similar reversal in spine increases, these results indicate that enhanced BDNF-TrkB signaling through either PLCγ or ERK is sufficient for this reversal. However, given that some degree of pERK is shared by TrkB-WT, TrkB-816/PLC, and TrkB-515/SHC (Fig. 2 *A–C*), it is possible that downstream ERK activation ultimately is sufficient to reverse spine formation induced by CSA. In any event, these findings indicate that BDNF-TrkB signaling can mediate bidirectional effects on the induction of new spine formation and their maintenance during and after chronic CSA.

Since increased BDNF-TrkB signaling in NACsh neurons reverses cocaine-induced morphological changes in spine density, we hypothesized that this effect may be associated with a reduction in addictive behavior. To test this hypothesis, animals

were subjected to a similar CSA regimen, and the effects of HSV vectors on subsequent CSA behavior were measured in sequential SA tests on fixed and progressive ratio (PR) reinforcement schedules (Fig. 2*K*, *Left*). Before HSV infusions, stabilized CSA was counterbalanced across the 5 study groups (Fig. 2*L*), and no group differences were apparent in baseline SA dose–response curves during within-session testing (Fig. S4*A*). Three days after intra-NACsh HSV infusions and during peak HSV-mediated expression, animals receiving the dominant negative TrkB-KD or TrkB-816/PLC vector showed a transient leftward shift in dose-sensitivity for maintaining CSA compared with GFP-expressing controls (Fig. 2*M*; effect of vector, $F_{4,39} = 3.389$, $P = 0.018$; effect of dose, $F_{4,156} = 67.0$, $P < 0.001$; interaction, $F_{16,156} = 2.133$, $P = 0.009$), an effect that recovered during the following week of testing (Fig. S4*B*), when HSV-expression is no longer evident (16). Rats were given an additional week of CSA to restabilize on the training dose, and no statistical differences in cocaine intake were evident (Fig. S4*C*). Rats subsequently received a second intra-NACsh infusion with the same HSV vector, and the degree of effort they would exert to maintain SA behavior was assessed on the PR reinforcement schedule. HSV-TrkB-KD or HSV-TrkB-816/PLC treatment enhanced the motivation for cocaine, as the number of cocaine injections earned increased (effect of vector, $F_{4,35} = 2.696$, $P = 0.047$; effect of dose, $F_{1,34} = 33.06$, $P < 0.001$), along with the highest ratio of lever-presses/cocaine injection achieved (Fig. 2*N*). Similar statistically significant increases in breakpoints at the 0.75 mg/kg injection dose were observed in a separate cohort after a single infusion (Fig. 2*K*, *Right*) of HSV-TrkB-KD or HSV-TrkB-816/PLC (Fig. S4*D* and Fig. 2*O*; $F_{2,11} = 6.203$; $P = 0.0157$), indicating a direct effect on CSA despite a lack of spine changes at a similar time of testing. Thus, transient loss of TrkB function via TrkB-KD after CSA enhanced cocaine reinforcement while not affecting cocaine-induced spine density in NACsh neurons (Fig. 2 *F–J*). Conversely, enhanced TrkB function produced by HSV-TrkB-WT reversed spine density increases without altering CSA behavior. Therefore, we conclude that these morphological and behavioral phenomena are not functionally related with respect to motivation for cocaine. These findings also indicate that selective loss of TrkB-PLCγ, but not TrkB-SHC-ERK, signaling conveys the motivational effects of global loss of TrkB function in NACsh neurons.

Given that gain of TrkB signaling (through PLCγ or ERK) reverses initial increases in spine density after chronic CSA, and loss of TrkB-PLCγ signaling enhances motivation for cocaine, we tested the effects of generalized PLCγ overexpression in NACsh on these morphological and behavioral measures. These experiments used a previously validated HSV-PLCγ vector that increases levels of pPLCγ to a greater extent than HSV-TrkB-WT in HEK293 cells (Fig. 3*A*) and also increases pERK (17) potentially due to downstream Ca^{2+} signaling cascades, as discussed earlier. Indeed, direct stimulation of PLCγ in HEK293 cells with the PLC activator m-3M3FBS increased pERK in a time-dependent manner (Fig. 3*B*) consistent with the notion that PLCγ can indirectly enhance pERK. This HSV-PLCγ vector showed prominent expression in NACsh neurons (Fig. 3*C*) and reversed increases in mushroom spines induced by chronic CSA (Fig. 3*D*) after 1 d of withdrawal to levels seen in saline SA controls (Fig. 3*E*; interaction: $F_{1,23} = 4.481$; $P = 0.0453$; Fig. S5). Taken together, these findings suggest reversal of cocaine-induced mushroom-shaped spines with gain of TrkB-PLCγ function ultimately may involve enhanced pERK signaling.

We next hypothesized that directly increasing PLCγ signaling would have an opposite effect on CSA behavior compared with the loss of TrkB-PLCγ signaling (Figs. 2 *M–O*). Rats were trained to SA cocaine as described earlier (Fig. 2*K*, *Left*), and subsequently tested in SA dose–response before (Fig. S4*E*), during (Fig. 3*H*; effect of dose, $F_{4,52} = 38.89$; $P < 0.001$), and after (Fig. S4*F*) HSV-mediated PLCγ overexpression in NACsh neurons. In contrast to loss of TrkB-PLC signaling, but similar to TrkB-WT, overexpression of PLC had no significant effect on CSA compared with GFP controls in dose–response tests on the fixed ratio schedule. After restabilization of CSA (Fig. S4*G*) and a second HSV injection, overexpression of PLCγ reduced the number of infusions earned on the PR reinforcement schedule at both doses [Fig. 3*I*; effect of vector, $F_{1,11} = 6.88$ ($P = 0.024$); effect of dose, $F_{1,13} = 14.48$ [$P = 0.002$]), and strongly depressed the final response/injection ratio achieved before cessation of SA behavior. The inability of HSV-PLCγ to reduce peak SA rates in fixed ratio testing indicates reduced PR responding was not related to a generalized suppression of operant behavior. The effect of enhanced PLCγ function on PR is opposite to the effect of eliminating TrkB-PLCγ signaling (Fig. 2 *N* and *O*). In addition, the ability of PLCγ to reduce CSA is discordant with the failure of TrkB-WT and TrkB-515/SHC to alter CSA, although all three vectors produce a similar reversal of cocaine-induced spine density in NACsh neurons.

Discussion

Chronic cocaine exposure increases spine density in NAC, and these morphological changes can persist for a month or more after withdrawal from repeated cocaine exposure (8). Dendritic spine formation is a hallmark of activity-dependent morphological changes in other brain regions, and BDNF-TrkB signaling is known to be a key player in these events (9). Here we found that local BDNF-TrkB signaling in NACsh neurons is necessary for the initial induction of spine density increases with repeated cocaine exposure. Thus, both inducible localized deletion of the TrkB gene, or limited transient expression of a dominant negative TrkB mutant, before the onset of repeated cocaine exposure prevented the formation of cocaine-induced thin spines without affecting basal density of any spine subtype. This loss of spine formation is associated with a reduction in mouse CSA behavior using similar constitutive deletion of either BDNF or TrkB in NAC neurons (2, 3). As CSA induces BDNF synthesis, release, and TrkB activation concomitant with each daily exposure (2, 3), it is likely that such dynamic BDNF-TrkB activity mediates the formation of new spines in the NACsh. In contrast, loss of TrkB function failed to alter spine increases after chronic CSA, suggesting ongoing TrkB activity is not necessary for maintaining newly formed spines, and probably is not necessary for spine maintenance after withdrawal from CSA. These findings suggest BDNF-TrkB signaling is necessary to maintain sensitivity to cocaine reinforcement, but argue against a functional relationship between spine growth and cocaine reinforcement.

However, we found that increasing TrkB function actually reversed cocaine-induced spines in NACsh neurons after their formation had occurred. This suggests BDNF-TrkB signaling is capable of mediating bidirectional effects on the induction and maintenance of morphological plasticity and potentially signifies an avenue for reversing relatively enduring structural changes in neurons due to prolonged chronic use of abused drugs. The reversal of cocaine-induced spines is consistent with BDNF's neurotrophic role that may unlock structural rigidity in NACsh neurons, a process we term cataplasticity (from Greek *cata*; reversal), possibly allowing for subsequent de novo synaptic alterations to occur. This process can be mediated by either TrkB-SHC signaling through the small GTPase Ras or TrkB-PLCγ signaling, but since both pathways converge downstream to increase pERK (10), ERK activation ultimately may mediate the reversal in cocaine-induced spines. CSA increases pERK in many brain areas, including the NACsh (18), and while ERK has a prominent role in mediating BDNF-induced spine formation in hippocampal neurons (9), there are reports of spine density reductions with similar sustained TrkB and ERK activity (19, 20). An alternative interpretation could be that TrkB reduces a different spine population from those induced by cocaine. This is

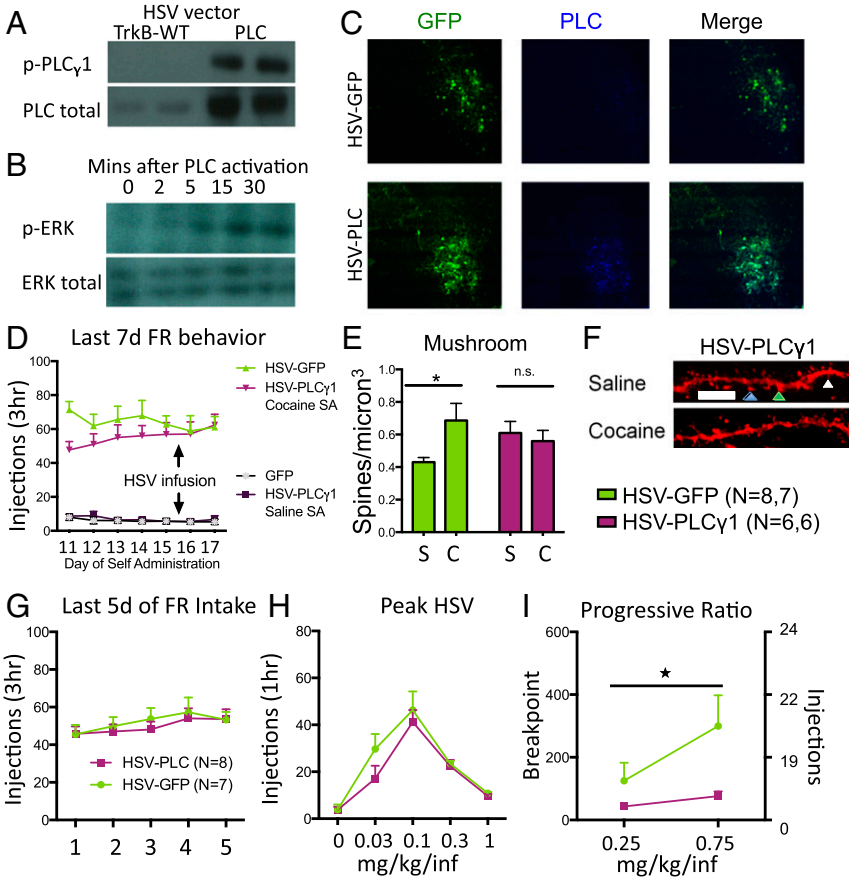

Fig. 3. PLCγ overexpression both reverses dendritic spines induced by chronic CSA and reduces CSA behaviors. (*A*) Western blot depicting increased phosphorylated and total PLCγ in HEK293 cells infected with HSV-PLCγ compared with HSV-TrkB-WT. (*B*) Time course of pERK after addition of the PLC activator m-3M3FBS (100 μM) over the course of 30 min. (*C*) Localized expression of GFP (green) and PLCγ (blue) 3 d after intra-NACsh infusions of HSV-GFP or HSV-PLCγ vectors. (*D*) Cocaine and saline SA rates before and after HSV-PLCγ or HSV-GFP infusions. (*E*) Quantification of mushroom spine density in saline and CSA overexpressing PLCγ or GFP. (*F*) Representative images of dendritic segments expressing HSV- PLCγ from saline and CSA animals. Representative spines are labeled white (thin), blue (mushroom), and green (stubby) to illustrate their categorization. (Scale bar, 10 μm.) (*G*) Average CSA (FR5) in study groups before (*H*) dose–response testing during peak HSV-mediated expression of PLCγ. (*I*) CSA on a PR schedule during GFP or PLCγ expression after a second HSV infusion (see Fig. 2*K*, *Left* for time line). The asterisk above a line represents a significant main effect of vector after a mixed factorial ANOVA. Data are expressed as mean ± SEM. $*P < 0.05$ compared with HSV-GFP controls. C, cocaine; n.s., not significant; S, saline.

unlikely, since no TrkB vector decreased basal spine density in saline animals, and only the subset of spines increased by cocaine (mushroom) was altered.

It is interesting that TrkB-mediated reversal of cocaine-induced dendritic spines is not accompanied by changes in sensitivity to low-dose cocaine reinforcement, or the effort rats exert to self-administer higher cocaine doses on the PR schedule. Instead, both dose sensitivity and the motivation for cocaine are enhanced by transient loss of TrkB function in NACsh neurons after chronic CSA. The effect on SA behavior clearly differs from constitutive and local knockout of TrkB in the NAC of cocaine self-administering mice discussed earlier (3). A similar enhancement in cocaine-seeking after early withdrawal is reported with viral expression of truncated TrkB or TrkB RNAi in the NAC core, but not shell, in more posterior NAc target regions in rats (5). These discrepancies in behavioral outcomes between TrkB knockout in mice, or knockdown of TrkB function in shell versus core in rats, may reflect species or subregional differences within the NACsh, but also could relate to gene deletion before cocaine versus functional knockdown after cocaine approaches. It also should be noted that our findings do not rule out potential effects of spine density on drug-seeking behavior in a drug-free state, as often measured in extinction/cue reinstatement tests.

Loss of TrkB-PLCγ, and not TrkB-SHC, signaling in NACsh increases CSA behaviors, suggesting the TrkB-PLCγ signaling pathway opposes addictive behavior. Moreover, the increase in motivation for cocaine with loss of TrkB-PLCγ is concurrent with reversal of cocaine-induced dendritic spines. However, since increased CSA is recapitulated with the dominant negative TrkB-KD that fails to alter spine density, this behavior probably reflects loss of other TrkB-PLCγ signaling events, rather than reversal of spine increases. In contrast, since a similar reversal of spine increases was found with TrkB-WT, the residual gain of TrkB-SHC-ERK function due to overexpression of the TrkB-PLCγ mutant likely explains the morphological effects. Contrary to the loss of TrkB-PLCγ signaling, overexpression of PLCγ itself is sufficient to reduce CSA behavior in PR reinforcement schedules. Thus, PLCγ signaling in NACsh neurons plays a pivotal role in regulating the motivation for cocaine reinforcement, and could represent a target for therapeutic intervention in the treatment of cocaine addiction. The specific mechanism of the anti-addictive effects of PLC is currently unknown, but could involve a direct interaction with the small GTPase Rac1 (21) that has a well-known role in spine morphology (22), or via Ca^{2+} signaling through protein kinase C that has been shown to alter drug conditioned place preference (23). Although overexpression of PLCγ also reverses cocaine-induced spines, other TrkB vectors

(TrkB-WT and TrkB-SHC) all produce a similar reversal of spine changes without altering CSA, and so it is difficult to attribute decreases in CSA with PLCγ overexpression to reversal of spine increases. In any event, the ability of PLCγ overexpression to increase pERK supports the notion that convergence on sustained downstream ERK activity ultimately mediates reversal of spine density increases, since all three TrkB vectors that decrease spine density also activate ERK. PLC can activate MEK-ERK signaling via Gab-1 or Ras-Raf mechanisms (10), but either TrkB-PLC or TrkB-SHC signaling also converges on other pathways, including PI3K, PKB, and Rac1, that may ultimately mediate these morphological effects (10).

Cocaine-induced increases in spine density can persist for several weeks after cessation of cocaine exposure (13, 24, 25), although chronic opiate exposure decreases spine density in the NAC (25), further questioning the relevance of these changes to maintenance of drug SA and vulnerability to relapse in withdrawal. Indeed, some investigators have suggested that increased spine density may represent a homeostatic counteradaptive response that diminishes drug sensitivity, or plays no role in locomotor sensitization to cocaine (26). One could speculate that spine formation reflects de novo generation of silent synapses in NAC neurons that ultimately recruit AMPA receptors after a prolonged period of withdrawal along with incubation of cocaine craving (27). In this sense, a BDNF-TrkB–mediated reversal of cocaine-induced spines could convey prominent anti-addictive properties, but only after prolonged abstinence. However, our prior work suggests daily CSA elevates transient BDNF-TrkB signaling in the NACsh, and not core (2, 3), where maturation of silent synapses and recruitment of synaptic AMPA receptors attenuates cocaine-seeking behavior (16, 28). Moreover, most studies show that dendritic spine increases revert to normal after about 1 mo from the last cocaine exposure (13). Thus, the role of spine density increases in drug addiction would be limited to perpetuating ongoing drug use, rather than promoting craving and relapse after long-term abstinence.

However, the present data refute a direct positive or negative relationship between such morphological changes and the motivation for cocaine, as TrkB-mediated reversal of sustained spine increases is not associated with changes in motivation for cocaine, loss of overall TrkB function enhances motivation for cocaine while leaving spine increases intact, and gain of PLCγ function reduces both behavioral and morphological measures. Given the multiple dissociations of morphological and behavioral effects in our study, we conclude that neither increases nor decreases in CSA behavior are mediated by alterations in total or mushroom-shaped spine density in the NACsh. Nevertheless, morphological cataplasticity is an attractive therapeutic target because treatments are administered after chronic cocaine use and reversion of spines to premorbid states could allow for new morphological responses to beneficial behavioral repertoires.

Methods

Complete methodology is available in the supplemental material. In brief, HSV vectors were constructed as previously described (29). In vitro HSV characterization was completed in HEK293 cells, and in vivo characterization was completed in NACsh tissue with Western blots using standard protocols (30) and IHC. CSA assays were performed similarly to previous methods (30). Dendritic spine analysis was performed with Volocity and Neuronstudio software similar to previously published methods (24). These studies were approved by the UTSW institutional Animal Care and Use Committee, and facilities are accredited by the American Association for the Accreditation of Laboratory Animal Care. All procedures were conducted in accordance with the guidelines established by the National Institutes of Health and the National Research Council.

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