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Tropomyosin-Related Kinase B in the Mesolimbic Dopamine System: Region-Specific Effects on Cocaine Reward

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Abstract

Background—Previous studies found that brain-derived neurotrophic factor (BDNF) derived from nucleus accumbens (NAc) neurons can mediate persistent behavioral changes that contribute to cocaine addiction.

Methods—To further investigate BDNF signaling in the mesolimbic dopamine system, we analyzed tropomyosin-related kinase B (TrkB) messenger RNA (mRNA) and protein changes in the NAc and ventral tegmental area (VTA) in rats following 3 weeks of cocaine self-administration. To study the role of BDNF-TrkB activity in the VTA and NAc in cocaine reward, we used localized viral-mediated Cre recombinase expression in floxed BDNF and floxed TrkB mice to knockdown BDNF or TrkB in the VTA and NAc in cocaine place conditioning tests and TrkB in the NAc in cocaine self-administration tests.

Results—We found that 3 weeks of active cocaine self-administration significantly increased TrkB protein levels in the NAc shell, while yoked (passive) cocaine exposure produced a similar increase in the VTA. Localized BDNF knockdown in either region reduced cocaine reward in place conditioning, whereas only TrkB knockdown in the NAc reduced cocaine reward. In mice self-administering cocaine, TrkB knockdown in the NAc produced a downward shift in the cocaine self-administration dose-response curve but had no effect on the acquisition of cocaine or sucrose self-administration.

Conclusions—Together, these data suggest that BDNF synthesized in either VTA or NAc neurons is important for maintaining sensitivity to cocaine reward but only BDNF activation of TrkB receptors in the NAc mediates this effect. In addition, up-regulation of NAc TrkB with chronic cocaine use could promote the transition to more addicted biological states.

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Keywords

Addiction; BDNF; nucleus accumbens; place preference; self-administration; TrkB

Drugs of abuse produce numerous cellular and molecular alterations that contribute to addiction-related behavioral change (1). Within the mesolimbic dopamine system, several drugs up-regulate the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling pathway leading to persistent activation of the transcription factor, cAMP response element binding protein (CREB) and of CREB-mediated gene expression (2,3). Cocaine-induced regulation of extracellular signal-regulated kinase (ERK) signaling also contributes to CREB activation (4,5). Among several drugs of abuse studied to date, cocaine most potently activates CREB in the ventral tegmental area (VTA) and nucleus accumbens (NAc), where a key molecular mediator of CREB's downstream effects is brain-derived neurotrophic factor (BDNF) (2,6-8).

Brain-derived neurotrophic factor messenger RNA (mRNA) is expressed at relatively low levels in the NAc but at considerably higher levels in VTA dopaminergic neurons and glutamatergic neurons (from several cortical and subcortical sites), both of which innervate the NAc (9-11). In contrast, the receptor for BDNF, tropomyosin-related kinase B (TrkB), is widely expressed in brain including the VTA and NAc (12,13). Brain-derived neurotrophic factor signaling via TrkB has been implicated in several forms of drug- and stress-induced plasticity (14-18). We recently found that transient increases in BDNF in the NAc shell during cocaine self-administration emanate from local synthesis in NAc neurons and promote persistent increases in drug-taking and drug-seeking behavior (19). In contrast, intra-NAc infusions of neutralizing anti-TrkB antibodies reduce psychostimulant-induced dopamine release and locomotor behaviors (20). Thus, while BDNF activation of TrkB receptors may facilitate dopamine release in the NAc, the relative contribution of postsynaptic TrkB receptors on medium spiny NAc neurons in addictive behavior is poorly understood.

In this study, we found that TrkB levels differentially increase in the VTA and NAc depending on the context of cocaine administration (volitional versus passive) in rats. We employed localized conditional knockout of BDNF and TrkB in VTA or NAc neurons in mice to determine the relative contribution of local BDNF synthesis and TrkB signaling in these regions on initial sensitivity to cocaine reward in place conditioning. We also studied the effect of localized TrkB knockout in NAc neurons on cocaine self-administration behavior.

Methods and Materials

Animals

Male Sprague-Dawley rats (275–325 g, Charles River, Kingston, Rhode Island), male floxed TrkB mice (21), and male floxed BDNF mice (14) were housed individually at 21°C (lights on: 0700–1900). All experiments were conducted in the light cycle and according to guidelines approved by the University of Texas (UT) Southwestern Institutional Animal Care and Use Committee.

Rat Cocaine Self-administration

To facilitate acquisition of cocaine self-administration, rats were maintained on a restricted diet at 85% original body weight and trained to press a lever for 45 mg sucrose pellets in operant chambers (Med Associates, Georgia, Vermont) on a fixed-ratio 1 (FR1) reinforcement schedule until acquisition criteria were achieved (100 pellets self-administered for 3 consecutive days). Rats were then fed ad libitum for at least 1 day prior to surgical intravenous catheterization as described previously and in Supplement 1 (22). Following 1 week of recovery, rats were trained

to self-administer intravenous cocaine hydrochloride (500 µg/kg/50 µL injection) or saline in daily 4-hour sessions for 3 weeks (6 days/week) (19). Self-administering rats were paired with yoked rats that received the same amount and temporal pattern of passive cocaine injections throughout training (chronic yoked). Another group received yoked saline injections on all but the final session, when they received cocaine injections for the first time (acute yoked). All cocaine and saline groups were compared with age- and group-matched home cage control rats that were handled daily. Cocaine self-administering rats developed stable responding by the beginning of the third week of training, and on the final training day (day 18), cocaine intake ranged from 25.5 mg/kg to 56.5 mg/kg among individual rats.

Immunoblotting and Reverse Transcription Polymerase Chain Reaction

To examine regulation of TrkB, tissue punches were dissected and reverse transcription polymerase chain reaction (RT-PCR) for mRNA performed as described in Supplement 1 using the following primer sequences: TrkB.FL (full length): 5'-GATCTTCACCTACGGCAAGC-3' & 5'-TCGCCAAGTTCTGAAGGAGT-3' (23), glyceraldehyde 3-phosphate dehydrogenase (GAPDH): 5'-AACGACCCCTTCATTGAC-3' & 5'-TCCACGACATACTCAGCAC-3' (19). For protein analysis, rats euthanized by microwave irradiation (5 kW, 1.5 sec, Murimachi Kikai Co., Tokyo, Japan) either immediately or 22 to 24 hours after the final test session. See Supplement 1 for additional details.

Region-Specific Gene Knockdown

Floxed BDNF or floxed TrkB mice, under ketamine/xylazine anesthesia, received bilateral stereotaxic adeno-associated virus (AAV) infusions of AAV-green fluorescent protein (GFP) or AAV-CreGFP (GFP fused to Cre recombinase) into the NAc or VTA using established stereotaxic coordinates and procedures (14,19): NAc (anterior-posterior (AP): 1.6 mm, medial-lateral (ML): ±1.5 mm, dorsal-ventral (DV): -4.4 mm, 10°) and VTA (AP: -3.2 mm, ML: ±1.0 mm, DV: -4.6 mm, 7°). The adeno-associated virus-2 (AAV-2) strain employed conveys selective expression in neurons with no potential for retrograde infection of afferent inputs to targeted brain regions, thereby limiting genetic deletion to local neuronal cell bodies (24,25).

Cocaine Conditioned Place Preference

Two weeks after viral injection, mice were tested for conditioned place preference to cocaine using an unbiased procedure in a three-chambered apparatus as described previously (14). On day 1 of place conditioning, mice received 30-min access to the conditioning apparatus to measure baseline compartment preference. On days 2 and 4, mice received an intraperitoneal cocaine injection (5 or 10 mg/kg) and were subsequently restricted to one side of the apparatus for 20 min. Alternatively, on days 3 and 5, mice received a saline injection that was paired with the other side of the apparatus. Preference was measured on day 6 by allowing mice free access to both compartments for a 30-min test session. Preference was calculated by subtracting the amount of time spent in the saline-paired environment from time in the drug-paired environment during the test session.

Mouse Sucrose and Cocaine Self-administration

Established protocols were employed to test mouse cocaine self-administration (19). Briefly, spontaneous lever-press behavior in the absence of reinforcement was measured in operant test chambers (Med Associates) in an initial 1-hour test in 16-hour food-restricted mice. On subsequent days, mice were tested for acquisition of sucrose pellet (25 g) self-administration (fixed-ratio 5 [FR5]) until 25 pellets were earned for 3 consecutive days (the third consecutive day was used for acquisition criteria). Mice were then fed ad libitum and surgically implanted with a chronic indwelling jugular catheter as described in Supplement 1. After at least 3 days recovery, acquisition of cocaine self-administration (500 µg/kg/50 µL injection) was tested in

daily 1-hour sessions on a FR1 (8-sec timeout) reinforcement schedule over 10 days and then gradually increased to FR5. Once intake was stable (15% variance for three consecutive sessions), mice were subsequently allowed to self-administer descending injection doses of cocaine, each for two consecutive daily 1-hour sessions, beginning with 1000 $\mu\text{g/kg}$ per injection and ending with saline; the number of cocaine injections and total cocaine intake from the second test at each dose were used for the analysis. Following successful completion of behavioral testing, viral expression sites were verified by GFP immunohistochemistry as described in Supplement 1.

Statistical Analysis

Levels of TrkB protein and mRNA were compared by oneway analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison tests. For cocaine place preference data, posttest preference scores for AAV-GFP and AAV-CreGFP infected animals were compared by unpaired *t* tests. Mouse self-administration data were analyzed by two-way ANOVA with repeated measures on dose or test session.

Results

Figure 1A depicts saline and cocaine treatment schedules for self-administering and yoked groups, with tissue collected either immediately after the final session (no withdrawal) or after 1 day withdrawal in the home cages. Chronic cocaine self-administration significantly increased full-length TrkB protein by 25% in the NAc shell (Figure 1B and 1C) but not the core (Table 1), when compared with untreated or saline self-administering control animals immediately following the last cocaine session. This increase was only apparent in actively self-administering rats and not yoked animals and returned to control levels after 1 day withdrawal. In contrast to the NAc, chronic cocaine self-administration did not alter TrkB protein levels in the VTA but a significant $\sim 30\%$ increase in TrkB was found in yoked animals that received cocaine passively with either acute or chronic administration (Figure 1B and 1D). Increases in TrkB protein were not accompanied by increased expression of TrkB mRNA in NAc or VTA (Table 1).

Infusion of an AAV vector encoding Cre recombinase into the NAc or VTA of floxed mice produced highly localized CreGFP expression in these brain regions (Figure 2A). We previously reported that NAc infusions of AAV-CreGFP in floxed BDNF mice produced a 46% reduction in BDNF protein in the NAc and an 80% reduction in the VTA (14,19). Similarly, we found that full-length TrkB protein levels were reduced by 25% in VTA tissue surrounding the infected region in floxed TrkB mice compared with AAV-GFP-infected control animals (Figure 2A). While this procedure infects $\sim 75\%$ of dopamine neurons in the VTA (26), it would expectedly infect nondopaminergic VTA neurons with similar efficiencies. Localized knockdown of BDNF in either NAc or VTA significantly reduced sensitivity to cocaine reward compared with AAV-GFP infected control animals in place conditioning with 10 mg/kg cocaine (Figure 2B). In contrast to BDNF, only localized deletion of TrkB receptors in NAc neurons significantly attenuated cocaine place conditioning, while TrkB deletion in VTA neurons was not effective (Figure 2C). There were no differences in baseline preference scores prior to conditioning among floxed BDNF and TrkB study groups, with mean scores ranging from 8.82 ± 24.2 sec to 15.0 ± 48.3 sec.

Since cocaine self-administration but not passive cocaine administration increased TrkB levels in the NAc shell, we tested the effects of localized TrkB knockdown in NAc neurons on cocaine self-administration behavior in floxed TrkB mice. Prior to reinforcement, both AAV-CreGFP (11.8 ± 6.3 responses) and AAV-GFP control animals (10.0 ± 3.4 responses) performed equivalent spontaneous lever-press behavior and subsequently acquired sucrose self-administration at similar rates (Figure 3A). Similarly, there was no effect of TrkB knockdown

on acquisition of cocaine self-administration on a FR1 reinforcement schedule (Figure 3B). However, after acquisition and stabilization on a FR5 schedule, localized TrkB knockdown in the NAc produced a downward shift in the inverted U-shaped dose-response curve, primarily at the peak of the curve (Figure 3C). Overall cocaine intake was also significantly reduced by localized deletion of TrkB in NAc neurons (Figure 3D).

Discussion

The effects of BDNF synthesized in NAc neurons can involve either activation of TrkB receptors on NAc neurons or binding to TrkB receptors on dopamine afferents, internalization, and retrograde transport to dopamine cell bodies in the VTA (27,28). Conversely, BDNF synthesized in VTA dopamine neurons can be released locally and act reciprocally on TrkB receptors to augment dopamine cell excitability (15) or undergo anterograde transport and release in the NAc to activate TrkB receptors on NAc neurons (9). In this study, we found that BDNF derived from either VTA or NAc neurons is important for maintaining initial sensitivity to cocaine reward, as indicated by a loss of cocaine place conditioning with localized BDNF deletion in either region. These findings are consistent with a recent report where BDNF and TrkB levels were modulated by lentiviral infusions in the NAc (29), but the lentivirus also modulates expression in VTA dopamine neurons by strong retrograde infection (30), so the effects cannot be localized to the infusion site. Importantly, our findings suggest that BDNF activation of TrkB receptors located specifically on NAc neurons is more important for maintaining sensitivity to cocaine reward than actions at TrkB receptors located on VTA neurons. These results establish a prominent role for TrkB receptors on NAc neurons in cocaine reward, but they do not entirely rule out a role for TrkB on VTA dopamine neurons, since TrkB also was deleted in nondopamine VTA neurons with local infusions.

Localized deletion of TrkB receptors on NAc neurons also led to a downward shift in the cocaine self-administration dose response curve, without producing a generalized impairment in instrumental learning capacity, and directionally opposite to changes that signal a transition to more addicted biological states (31). This effect is remarkably similar to the effect of localized deletion of BDNF in NAc neurons in our previous study (19). Together, these results indicate that BDNF induction and release with cocaine use is at least partially derived from local synthesis in NAc neurons (19) and acts reciprocally on TrkB receptors expressed by NAc neurons to promote cocaine reinforcement and the development of cocaine addiction (present findings). Furthermore, given that chronic cocaine self-administration produced a reinforcement-related up-regulation in TrkB receptors in the NAc shell of rats, this neuroadaptation could facilitate the transition to cocaine addiction by augmenting the response to transient BDNF activity during active cocaine self-administration. However, TrkB mRNA failed to increase in the NAc, potentially reflecting limitations of RT-PCR or that cocaine-induced increases in TrkB protein in the NAc could possibly be derived from cortical or VTA afferent terminals in the NAc. In addition, it is not known whether cocaine self-administration would increase TrkB in the NAc shell of mice where TrkB was shown to modulate cocaine self-administration.

Interestingly, passive yoked but not self-administered cocaine increased TrkB in the VTA, even with a single acute 4-hour exposure. This increase in TrkB protein was also not accompanied by increases in TrkB mRNA and potentially emanates from afferent inputs as discussed above. Otherwise, TrkB could increase via nontranscriptional mechanisms including increased translation, protein stability, or reduced degradation in VTA (or NAc shell) neurons. Increased TrkB in the VTA of yoked animals potentially results from the stressful effects of uncontrollable intravenous cocaine injections and highlights the fact that self-administered cocaine often produces different neurobiological changes than experimenter-administered cocaine treatment regimens. Along these lines, chronic social defeat stress is mediated, in part,

by BDNF expressed in VTA, but not NAc, neurons (14). In addition, TrkB up-regulation with yoked cocaine could explain the failure of TrkB knockdown in the VTA to reduce cocaine reward with similar passive cocaine administration in place-conditioning tests. Thus, TrkB up-regulation in non-AAV-infected VTA neurons potentially could counteract the effects of genetic deletion in AAV-infected neurons. Given the fact that local BDNF activation of TrkB receptors potentiates VTA dopamine cell excitability in cocaine withdrawal (15) and midbrain infusions of BDNF enhance forebrain dopamine release (32), further experimentation, perhaps employing gain of TrkB function approaches, is needed before the role of TrkB in the VTA in cocaine reward can be ruled out.

Our findings support the notion that therapeutic strategies aimed at neutralizing BDNF-TrkB receptor signaling in the NAc may have utility when given during active cocaine self-administration as suggested previously (19). These data also suggest that reduced vulnerability to drug abuse in people with a single nucleotide valine 66/methionine (met) polymorphism in the BDNF gene may be related to impairments in BDNF release (33,34). Thus, we recently reported that BDNF met66 mice show reduced activity-dependent BDNF signaling within the VTA-NAc circuit (14), which may result in an attenuated ability of drugs to induce addiction-promoting pathological changes in the NAc. Given that TrkB receptors activate multiple signaling cascades with differential modulation of neuroplasticity and cellular morphology (35), future studies should identify which of these events contribute to escalation of cocaine intake and a propensity for relapse induced by local BDNF-TrkB activation in NAc neurons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Volitional and passive cocaine administration differentially regulates TrkB in the NAc shell and VTA. **(A)** Schematic figure depicting timelines and treatment groups for self-administering and yoked rats used for TrkB protein and mRNA determinations. Rats self-administered cocaine (500 μ g/kg/50 μ L injection) or saline in daily 4-hour sessions over 18 days (6 days/week), and tissue was collected immediately after the final session or after 1-day withdrawal (WD). Self-administering rats were paired with yoked rats that received the same amount and temporal pattern of passive cocaine injections throughout training (chronic yoked). Another group received yoked saline injections on all but the final session, when they received cocaine injections for the first time (acute yoked). **(B)** Representative blots depicting TrkB protein

changes in the NAc shell or VTA with acute yoke (AY), chronic yoke (CY), and chronic cocaine SA (CSA) compared with untreated home cage control rats (HC). **(C)** TrkB protein levels increase in the NAc shell immediately after 4 hours of CSA (SA) but not in yoked animals [$F(3,156) = 6.47, p < .001$] and returns to basal levels 24 hours later. **(D)** In contrast, in the VTA, TrkB protein levels increase in cocaine yoke (acute and chronic) but not in cocaine self-administering animals immediately after the session [$F(3, 116) = 11.31, p < .001$]. These data indicate that the context of cocaine reinforcement is important for TrkB regulation in the NAc shell, while the stressful effects of unanticipated cocaine injections in yoked animals potentially contribute to regulation of TrkB in the VTA. Animals self-administering saline throughout show no statistically significant difference in TrkB levels compared with untreated HC control animals. Data are expressed as mean $\pm$ SEM percent change from HC control animals. Asterisk indicates $p < .05$ compared with pooled HC and saline SA control animals (cocaine-exposed: $n = 6-9$, untreated and saline SA control animals: $n = 8-24$). AY, acute yoke; CY, chronic yoke; CSA, cocaine self-administration; HC, home cage control rats; mRNA, messenger RNA; NAc, nucleus accumbens; SA, self-administering; TrkB, tropomyosin-related kinase B; VTA, ventral tegmental area; WD, withdrawal.

Figure 2.

Effects of BDNF and TrkB deletion in VTA and NAc neurons on cocaine reward. **(A)** Representative GFP immunofluorescence depicting localized AAV-mediated GFP-Cre expression in medial NAc and VTA neurons. Immunoblots from VTA tissue infected with AAV-CreGFP show a 25% reduction in full-length TrkB protein levels compared with AAV-GFP infected control animals ($n = 3$ mice/group). **(B)** Localized BDNF knockdown in either VTA neurons [$t(28) = 2.244, p < .05$] or NAc neurons [$t(24) = 2.067, p < .05$] reduces cocaine place conditioning (10 mg/kg). **(C)** In contrast, only localized TrkB knockdown in NAc neurons [$t(17) = 2.180, p < .05$], but not VTA neurons, reduces cocaine place conditioning. Data are expressed as mean $\pm$ SEM difference scores for time spent in drug- and saline-paired

sides. Asterisk indicates $p < .05$ compared with AAV-GFP control animals by unpaired t test ($n = 8-17$ mice/group). AAV, adeno-associated virus; BDNF, brain-derived neurotrophic factor; Cre, Cre recombinase; CreGFP, green fluorescent protein fused to Cre recombinase; GFP, green fluorescent protein; NAc, nucleus accumbens; TrkB, tropomyosin-related kinase B; VTA, ventral tegmental area.

Figure 3.

Effects of TrkB deletion in NAc neurons on sucrose and CSA. **(A,B)** Localized TrkB knockdown in NAc neurons (AAV-CreGFP) has no effect on the rate of acquisition of lever-press behavior for sucrose pellets (FR5) or CSA (FR1; 500 μg/kg/injection) compared with control mice (AAV-GFP). **(C)** However, after acquisition, localized TrkB knockdown in NAc neurons produces a downward shift in the inverted U-shaped dose-response curve for CSA (FR5) compared with control mice [group effect, $F(1,70) = 5.44$, $p < .001$]. **(D)** Conversion of dose-response data into dose intake (# of injections × unit dose/injection, cocaine only) shows an overall reduction in cocaine intake [group effect, $F(1,70) = 11.64$, $p < .01$]. Data are expressed as **(A)** a percentage meeting acquisition criteria or **(B–D)** mean ± SEM

reinforcements/hour ($n = 6-7$ mice/group). AAV, adeno-associated virus; CreGFP, green fluorescent protein fused to Cre recombinase; CSA, cocaine self-administration; FR1, fixed-ratio 1; FR5, fixed-ratio 5; GFP, green fluorescent protein; NAc, nucleus accumbens; TrkB, tropomyosin-related kinase B.

Table 1
Negative Regulation of TrkB Protein and mRNA with No Withdrawal

Study Group	TrkB Protein	TrkB mRNA		
	NAc Core	NAc Core	NAc Shell	VTA
Home Cage	0 ± 6.0	NT	NT	NT
Saline SA	-2.3 ± 5.1	0 ± 9.3	0 ± 9.3	0 ± 8.9
Acute Yoke	6.5 ± 6.3	NT	NT	NT
Chronic Yoke	3.8 ± 4.0	0 ± 9.7	0 ± 9.3	9 ± 8.8
Cocaine SA	7.9 ± 4.2	15 ± 9.4	-13 ± 9.5	29 ± 9.3

Values indicate % Δ from home cage or saline self-administering (SA) control animals.

NAc, nucleus accumbens; mRNA, messenger RNA; NT, not tested; SA, self-administering; TrkB, tropomyosin-related kinase B; VTA, ventral tegmental area.