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Accumbens brain-derived neurotrophic factor (BDNF) transmission inhibits cocaine seeking

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Abstract

Brain-derived neurotrophic factor (BDNF) regulates a variety of physiological processes, and several studies have explored the role of BDNF in addiction-related brain regions like the nucleus accumbens core (NAcore). We sought to understand the rapid effects of endogenous BDNF on cocaine seeking. Rats were trained to self-administer cocaine and extinguished. We then microinjected two inhibitors of BDNF stimulation of tropomyosin receptor kinase B (TrkB), the non-competitive receptor antagonist ANA-12 and TrkB/Fc, a fusion protein that binds BDNF and prevents TrkB stimulation. Blocking TrkB or inactivating BDNF in NAcore potentiated active lever pressing, showing that endogenous BDNF tone was present and supplying inhibitory tone on cue-induced reinstatement. To determine if exogenous BDNF also negatively regulated reinstatement, BDNF was microinjected into NAcore 15 minutes before cue-induced reinstatement. BDNF decreased cocaine seeking through TrkB receptor binding, but had no effect on inactive lever pressing, spontaneous or cocaine-induced locomotion, or on reinstated sucrose seeking. BDNF-infusion potentiated within trial extinction when microinjected in the NAcore during cue- and context + cue induced reinstatement, and the inhibition of lever pressing lasted at least 3 days post injection. Although decreased reinstatement endured for 3 days when BDNF was administered prior to a reinstatement session, when microinjected before an extinction session or in the home cage, BDNF did not alter subsequent cued-reinstatement. Together, these data show that endogenous BDNF acts on TrkB to provide inhibitory tone on reinstated cocaine seeking, and this effect was recapitulated by exogenous BDNF.

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Authors contribution

A.-C.B., C.G.-K., V.C., J.H. and D.M.C. performed the experiments. J.A.H. participated in conceptual discussions. A.-C.B. and P.W.K. developed the study and wrote the manuscript. All authors reviewed the final manuscript.

Disclosure

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Keywords

BDNF; cocaine seeking; nucleus accumbens; rats; self-administration; sucrose-seeking

INTRODUCTION

Cocaine addiction is a chronic disorder that is characterized by high vulnerability to relapse in response to cues or contexts associated with drugs, even after long periods of abstinence (Sinha 2011). One key region for initiating cue-induced cocaine seeking is the nucleus accumbens core (NAcore). The NAcore receives glutamatergic projections from the prelimbic cortex (PL) that are necessary for cocaine-induced (Capriles *et al.* 2003), context-induced (Fuchs *et al.* 2007), stress-induced (Capriles *et al.* 2003) and cue-induced (Stefanik, Kupchik, & Kalivas 2016) reinstatement of cocaine-seeking in animal models of relapse.

Synaptic plasticity within the glutamatergic PL-NAcore pathway plays a central role in reinstatement (Mulholland, Chandler, & Kalivas 2016), and brain-derived neurotrophic factor (BDNF) modulates activity-dependent plasticity involved in cocaine-seeking (Li & Wolf 2015; Koskela *et al.* 2016). Abstinent cocaine addicts show a correlation between serum BDNF and relapse risk (D'Sa *et al.* 2011; Corominas-Roso *et al.* 2015). Similarly, single and repeated non-contingent cocaine injections (Le Foll, Diaz, & Sokoloff 2005; Filip *et al.* 2006; Liu *et al.* 2006; Fumagalli *et al.* 2007) or self-administered cocaine (Graham *et al.* 2007; Sadri-Vakili *et al.* 2010; Schmidt *et al.* 2012; Li *et al.* 2013) increase BDNF mRNA and protein levels in the prefrontal cortex (PFC), nucleus accumbens (NAcc) and ventral tegmental area (VTA). Other studies also show time-dependent increases of BDNF levels in the VTA, NAcc and amygdala associated with the incubation of drug craving (Grimm *et al.* 2003). Cocaine-induced changes in BDNF-TrkB signaling are similarly observed when cocaine is administered at prenatal (McCarthy *et al.* 2016) or early developmental stages (Giannotti *et al.* 2014). Also, contingent cocaine increases the expression of the primary BDNF receptor, tropomyosin receptor kinase B (TrkB) in the shell subregion of the NAcc (NAshell) (Graham *et al.* 2009).

Consistent with a positive relationship between cocaine exposure and BDNF levels, repeated BDNF infusions into the VTA or NAcc increase cocaine-induced behavioral sensitization (Horger *et al.* 1999; Pierce, Pierce-Bancroft, & Prasad 1999) and self-administration (SA), and neutralizing BDNF in the NAcc reduces SA (Graham *et al.* 2007). Moreover, virus-mediated deletion of TrkB in NAcc reduces cocaine SA and cocaine conditioned place preference (CPP) (Graham *et al.* 2009), and virus-mediated over expression of TrkB in the NAshell regulates chronic cocaine-induced increases in spine density; although, this morphological regulation is unrelated to rates of cocaine SA (Anderson *et al.* 2017). Finally, systemic administration of a brain-penetrant TrkB antagonist reduces cocaine intake, motivation and reinstatement of cocaine seeking (Verheij *et al.* 2016).

BDNF also modulates context-induced and cue-induced cocaine seeking (Barker *et al.* 2015; Li *et al.* 2015). A single BDNF injection into the PFC activates the ERK signaling pathway, changes the phosphorylation of NMDA receptors in the PL by activating Src family kinases (Barry & McGinty 2017) and produces enduring inhibition through normalizing cocaine-

mediated extracellular glutamate spillover in the NAcc (Berglind *et al.* 2009). In contrast, acute BDNF microinjection into the VTA promotes context-induced and cue-induced cocaine seeking through activation of the MAPK pathway (Lu *et al.* 2004). While BDNF is produced locally in the NAcc (Graham *et al.* 2007), BDNF is also anterogradely transported to the striatum from the PFC and VTA (Altar & DiStefano 1998), posing the possibility that BDNF release may differentially regulate reinstatement depending on the anatomical source to the NAcc. Other studies report differential effects of BDNF on relapse between the core and the shell subregions of the NAcc (Li *et al.* 2013). Differential regulation in NAcc is indicated by the fact that although the TrkB gene is expressed in both D1- and D2-receptor expressing medium spiny neurons (MSNs) (Freeman, Soghomonian, & Pierce 2003), BDNF differentially regulates cocaine CPP via selective D1- or D2-MSN TrkB activation (Lobo *et al.* 2010).

The studies outlined above examining enduring manipulations of BDNF or TrkB levels likely involved the well-established transcriptional regulation produced by BDNF. Here, we primarily focused on the acute, non-transcriptional effects of endogenous and exogenous BDNF in the NAcc on cue-induced reinstatement after cocaine SA, but also longer lasting effects in subsequent reinstatement testing. We found that both endogenous and exogenous BDNF in NAcc inhibit cue-induced cocaine seeking by stimulating TrkB, and this effect arises from accelerating within reinstatement trial extinction of lever pressing. The inhibitory effect of acute BDNF on cued reinstatement endured for at least 3 days, but only if BDNF was microinjected in conjunction with a reinstatement session, indicating that the enduring inhibition required BDNF in conjunction with activating cue-associated memories.

MATERIAL AND METHODS

Animals and surgery

Male Sprague Dawley rats (200–250 g, Charles River Laboratories) were housed individually in a temperature-controlled environment on a 12-hour reverse light cycle. After 1 week of acclimation, animals were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (7 mg/kg, i.p.) and implanted with indwelling jugular catheter and bilateral 26 gauge cannulae (PlasticsOne, VA) were inserted targeting the NAcc (+1.5 A/P, +1.7 M/L, –5.5 D/V), secured in place with dental cement and maintained open with 0.009" × 30" internal stylets. Rats undergoing sucrose SA were only implanted with bilateral cannulae. All rats received ketorolac analgesic (0.28–0.32 mg/kg) and prophylactic antibiotic (Cefazolin, 200 mg/ml, subcutaneous; West-Ward Pharmaceuticals, NJ). Animals had 1 week to recover from surgery before starting behavioral training.

Reagents

All reagents were dissolved in 1 percent DMSO in saline. Vehicle (VEH) refers 1 percent DMSO in saline. Human BDNF (Peprotech, Rocky Hill, NJ, USA) was microinjected at 2 µg/µl (i.e. 1 µg/0.5 µl/side), a dose within the order of magnitude used in the literature (0.24–2.5 µg/0.5 µl/side) (Lu *et al.* 2004; Graham *et al.* 2007; Berglind *et al.* 2009). The TrkB selective and non-competitive antagonist ANA-12 (SML0209, Sigma-Aldrich, St Louis, MO, USA) (Cazorla *et al.* 2011) was microinjected in combination with BDNF or alone at

0.2 pg/ μ l (i.e. 0.1 pg/0.5 μ l/side (Shirayama *et al.* 2015). We also tested a higher dose of [ANA-12] = 2 pg/ μ l (i.e.1 pg/0.5 μ l/side), but this dose decreased spontaneous locomotion (data not shown). Human recombinant TrkB/Fc chimera (T8694; Sigma-Aldrich, St Louis, MO, USA), a soluble scavenger form of the TrkB receptor, was microinjected in combination with BDNF or alone at 1.3 μ g/ μ l (i.e. 650 ng/0.5 μ l/side) (Revest *et al.* 2014).

Cocaine self-administration (SA) procedure

Two days before starting cocaine SA, rats were food deprived overnight (22 g chow) and food trained under a FR1 reinforcement schedule to press for chow (45 mg, Bio-Serv, Flemington, NJ) for a single 2-hour session in standard rat modular test chambers (Med Associates, Fairfax, VT). Food training facilitated cocaine SA acquisition. Cocaine SA consisted of daily 2-h sessions on FR1 reinforcement schedule during which active lever presses resulted in cocaine delivery (0.25 mg/ infusion, NIDA) paired with a compound cue (light and tone, 78 dB, 4.5 kHz). Programs integrated a 20-second time out and a maximum of 80 infusions per session to prevent cocaine overdose. Following a minimum of 10 days of 10 infusions or more, rats entered extinction training, during which time lever presses had no consequences. Extinction criterion was reached when the average of active lever presses during the last 3 days of extinction was equal or less than 30 percent of the average of the last 3 days of SA, which usually happened after 10–12 days of extinction training. One group of animals underwent forced abstinence. Acquisition of SA was identical to extinguished animals, but instead of extinction training, these animals stayed in their home cage and were handled daily to prevent handling bias.

Sucrose self-administration (SA) procedure

Sucrose SA consisted of daily 2-hour sessions in standard rat modular test chambers (Med Associates, Fairfax, VT) on an FR1 reinforcement schedule during which active lever presses resulted in delivery of a sucrose pellet (45 mg, Bio-Serv, Flemington, NJ) paired with a compound cue (light and tone, 78 dB, 4.5 kHz). Following a minimum of 10 days of 10 pellets or more, rats entered extinction training, during which time lever presses had no consequences. Extinction criterion was reached, and animals were tested when the average of active lever presses during the last 3 days of extinction was equal or less than 30 percent of the average of the last 3 days of SA, which usually happened after 10–12 days of extinction training. Animals were maintained on food restriction (22 g chow per day) throughout behavioral testing. Behavioral sessions were carried out in the afternoon.

Reinstatement, microinjections and histology

Cocaine and sucrose 2-hour reinstatement tests were induced by contingent exposure to the cues previously paired with cocaine or sucrose, but without reward delivery. Active lever presses were interpreted as cocaine or sucrose seeking. For animals undergoing forced abstinence, cocaine seeking was induced by reintroducing rats in the chamber previously associate with cocaine delivery and presenting contingent cues, without any cocaine reward.

Rats were microinjected 2 mm below the cannula tip using 33-gauge microinjectors at 0.25 μ l/min for a total volume of 0.5 μ l per hemisphere. Microinjectors were left in place to allow diffusion for 2 minutes. Animals returned to home cage for 15 additional minutes before

starting reinstatement sessions to allow recovery from the microinjection procedure. For all experiments, rats are tested as between-subjects and there are no counter-balanced crossovers due to the long-lasting effects of BDNF. At the end of each experiment, anesthetized rats were transcardially perfused with 1X PBS followed by a 10 percent phosphate buffered formalin solution. Brains were sliced at 100 μm on a vibratome and stained with cresyl violet to verify cannula placement (Fig. S1B).

Locomotor activity

The effect of reagents on spontaneous locomotion and cocaine-induced locomotion was performed on the same animals that underwent cocaine self-administration and reinstatement procedures included in Figure 2a–c, in a randomized fashion controlling for previous treatment. Rats were microinjected with BDNF, ANA-12 or TrkB/Fc followed by 15-minute recovery in house cage, followed by a 1-hour open field session in the Digiscan Animal Activity Monitor system (Omnitech Electronics Model RXYZCM, Columbus, OH). For cocaine-induced locomotion, BDNF was infused in the NAcore 15 minutes before administering 20 mg/kg cocaine i.p. and recording the locomotion for 1 hour.

Experimental design and statistical analysis

For comparing multiple measurements in the same experiment, the data were analyzed using two-way ANOVA as indicated in the text describing each experiment. Sidak's post hoc test was applied for multiple comparisons, and $P < 0.05$ was considered statistically significant. Locomotor activity comparison between vehicle or BDNF infused rats were analyzed using unpaired Student's t-tests. All statistical tests were conducted using the Prism (Graphpad, La Jolla, CA) software package.

RESULTS

Cocaine self-administration (SA)

During the acquisition of SA, rats quickly learned to self-administer cocaine and discriminated between the active and the inactive lever by more than 80 percent at the end of acquisition (Fig. S1A). The number of cocaine infusions remained stable throughout SA (~35 infusions/2 hour session). After reaching SA criteria of 10 days with 10 infusions per day and more than 70 percent discrimination between active and inactive levers, rats underwent extinction training (Fig. S1A), during which active lever presses were not rewarded by cocaine or cues. After a burst of pressing on extinction day 1 rats rapidly reduced pressing both levers (Fig. S1A). Once active lever pressing in extinction reached <30 percent of the mean of the last 3 days of cocaine SA during acquisition, cue-induced cocaine seeking was tested. Figure S1B shows a representative micrograph, and Figure S1C shows cannula tip placements for all animals included in the experiments described below, divided by experiment for clarity.

Endogenous BDNF applies inhibitory tone on cued-reinstatement

To determine if there is endogenous tone by BDNF on TrkB in the NAcore that is consequential in cued-reinstatement of cocaine seeking, we microinjected one of two different inhibitors of BDNF stimulation of TrkB into the NAcore 15 minutes before cued-

reinstatement. A two-way ANOVA for active lever revealed effects of extinction/reinstatement $F_{(1,21)} = 77.12$, $P < 0.0001$, treatment $F_{(2,21)} = 5.57$, $P = 0.012$ and an interaction $F_{(2,21)} = 4.86$, $p = 0.019$ (Fig. 1a). A Sidak's post hoc comparison revealed a significant increase of active lever presses in the presence of cues compared to extinction for all groups. Moreover, microinjection of the TrkB non-competitive antagonist ANA-12 (0.1 pg/0.5 μ l/side) significantly potentiated cued reinstatement compared to control. In contrast to ANA-12, there is no potentiation of cocaine seeking after microinjection of a TrkB receptor scavenger, the human recombinant TrkB/Fc chimera. However, when the time course of cue-induced active lever pressing was examined, both ANA-12 and TrkB/Fc augmented the first 15 minutes of cue-induced active lever pressing (Fig. 1b; two-way ANOVA with repeated measures over time for active lever revealed effects of treatment $F_{(2,21)} = 5.86$, $P = 0.001$, time $F_{(7,147)} = 19.50$, $P < 0.0001$ and interaction $F_{(14,147)} = 2.33$, $P = 0.006$). A Sidak's post hoc comparison revealed ANA-12 microinjection significantly increased active lever pressing compared to control at 15 minutes and 90 minutes, while TrkB/Fc potentiated lever pressing at 15 minutes. There was no effect on inactive lever pressing by either TrkB antagonist (Fig. S2, a two-way ANOVA for inactive lever revealed no significant effects of extinction/reinstatement $F_{(1,21)} = 0.01$, $P = 0.939$, treatment $F_{(2,21)} = 0.49$, $P = 0.6$ or interaction $F_{(2,21)} = 0.67$, $P = 0.524$). These results indicate that endogenous BDNF provides inhibitory tone on cue-induced cocaine seeking.

Acute exogenous BDNF in the NAc core recapitulates endogenous BDNF

An acute infusion of BDNF (1 μ g/0.5 μ l/side) into the NAc core 15 minutes before cue-induced reinstatement reduced cocaine seeking by ~50 percent (Fig. 2a). BDNF-induced reduction in active lever pressing was not different from extinction level pressing. A two-way ANOVA for active lever revealed effects of extinction/reinstatement $F_{(3,37)} = 55.54$, $P < 0.0001$, treatment $F_{(3,37)} = 3.04$, $P = 0.041$ and interaction $F_{(3,37)} = 3.21$, $P = 0.034$. A Sidak's post hoc comparison established a significant potentiation of active lever pressing in the presence of cues compared to extinction for animals microinjected with vehicle, ANA-12 + BDNF and TrkB/FC + BDNF, but not in the BDNF microinjected group. The post hoc test also revealed a significant inhibition of lever pressing in the presence of cues in the BDNF group compared to the vehicle control. A two-way ANOVA of inactive lever pressing revealed no significant effect of extinction/reinstatement $F_{(1,37)} = 0.46$, $P = 0.5$, treatment $F_{(3,37)} = 0.76$, $P = 0.524$ or interaction $F_{(3,37)} = 2.82$, $P = 0.052$ (Fig. S3).

To determine if the inhibition of cued reinstatement by BDNF was mediated by TrkB, we microinjected a combination of BDNF and TrkB antagonist ANA-12 (0.1 pg/0.5 μ l/side) (Fig. 2a). ANA-12 prevented the BDNF-induced decrease in cue-induced cocaine seeking, and restored levels of active lever pressing to vehicle treatment levels. To assure the acute role of TrkB in BDNF-induced decrease of cocaine seeking, we also blocked TrkB signaling by microinjecting TrkB/Fc in combination with the BDNF microinjection. Similar to ANA-12, TrkB/Fc + BDNF microinjected rats showed a significant increase in cocaine seeking during cued-reinstatement. There was no effect of the combination of BDNF + ANA-12 or BDNF + TrkB antagonist on inactive lever pressing (Fig. S3).

BDNF seemed to potentiate within session extinction of the cues, since a steep decrease of active lever pressing occurred between 15 and 30 minutes in the cued-reinstatement session, whereas VEH microinjected animals show a more gradual decrease over time (Fig. 2b). A two-way ANOVA for active lever revealed effects of time $F_{(7,259)} = 8.91, P < 0.0001$, treatment $F_{(3,37)} = 4.34, P = 0.010$ but no significant interaction $F_{(21,259)} = 1.29, P = 0.181$. Figure 2c shows the time course of active lever pressing during cue-induced reinstatement in animals receiving microinjection of vehicle, a combination of ANA-12 + BDNF or TrkB + BDNF. The vehicle group is the same as in Figure 2b, the four treatment groups being divided between Figure 2b and c for clarity. The statistical analysis was performed jointly.

The inhibitory effect of acute exogenous BDNF is specific to cocaine seeking

Cocaine seeking measured during cued-reinstatement is a combination of complex behaviors including locomotion and motivation associated with non-drug rewards. In order to isolate the effect of BDNF on motivated seeking for cocaine reward, we tested the effect of BDNF on locomotion and cue-induced sucrose seeking. Acute BDNF microinjection in the NAcore 15 minutes before introducing rats to a new open field did not decrease exploratory behavior (Fig. 3a; an unpaired Student's test revealed no differences between groups, $t(17) = 1.53, P = 0.140$), and did not reduce locomotion induced by cocaine (20 mg/kg, ip) (Fig. 3a; $t(16) = 0.86, P = 0.40$). We additionally tested spontaneous locomotion in a novel open field after ANA-12 and TrkB/Fc microinjections into the NAcore, and neither drug altered locomotion compared to vehicle (Fig. 3b). A one-way ANOVA revealed no differences between groups, $F_{(2,26)} = 0.43, P = 0.65$.

To determine if BDNF inhibited seeking of a natural reward, a group of rats underwent sucrose SA, extinction training and cued-reinstatement (Fig. 3c–e). An acute microinjection of BDNF in the NAcore 15 minutes before cue-induced reinstatement did not modify sucrose seeking (Fig. 3d), suggesting that BDNF-mediated inhibition was related to adaptations produced by cocaine that were not also induced by sucrose. A two-way repeated measures ANOVA revealed an effect of extinction/cue $F_{(1,20)} = 38.80, P < 0.0001$, but no effect of treatment $F_{(1,20)} = 0.58, P = 0.456$ or interaction $F_{(1,20)} = 2.01, P = 0.171$. A two-way ANOVA revealed no effect by BDNF on inactive lever pressing for extinction/cue $F_{(1,20)} = 2.67, P = 0.118$, treatment $F_{(1,20)} = 0.15, P = 0.703$ or interaction $F_{(1,20)} = 0.91, P = 0.352$ (Fig. S4). The time course of cued-reinstatement to sucrose (Fig. 3e) shows similar within session extinction of sucrose-paired cues in vehicle- or BDNF-microinjected animals. Although BDNF-treated group shows a decreased active lever pressing at 15 minutes within the cue-induced sucrose reinstatement, a two-way repeated measures ANOVA for active lever presses revealed an effect of time $F_{(7,140)} = 11.07, P < 0.001$, but no significant effect of treatment $F_{(1,20)} = 1.3, P = 0.269$ or interaction $F_{(7,140)} = 0.88, P = 0.521$.

Acute exogenous BDNF in the NAcore potentiates within session extinction during context + cue-induced reinstatement after forced abstinence

BDNF inhibited cocaine seeking in rats that underwent extinction training after cocaine SA. It was possible that extinction training was necessary for the actions of BDNF. Extinction training produces enduring changes in the NAcc compared to rats or mice placed in abstinence for an equivalent period without extinction training (Sutton *et al.* 2003;

Knackstedt *et al.* 2010). Accordingly, we infused BDNF in the NAcore 15 minutes before context + cue-induced cocaine seeking after 12–14 days of abstinence without extinction training. We found no decrease in active (Fig. 4a) or inactive (Fig. S5) lever pressing over the 2-hour session in BDNF-infused rats; a two-way repeated measures ANOVA for active lever presses revealed an effect of last day of SA versus context + cue-induced reinstatement $F_{(1,16)} = 8.12$, $P = 0.012$, but no effect of treatment $F_{(1,16)} = 2.62$, $P = 0.125$ or interaction $F_{(1,16)} = 0.08$, $P = 0.783$). A two-way repeated measures ANOVA for inactive lever presses revealed an effect of last day of SA versus context + cue-induced reinstatement $F_{(1,16)} = 32.62$, $P < 0.001$, but no effect of treatment $F_{(1,16)} = 0.60$, $P = 0.451$ or interaction $F_{(1,16)} = 3$, $P = 0.103$ (Fig. S5).

BDNF after forced abstinence produced highly variable results ranging from complete inhibition of cocaine seeking to no effect on cocaine seeking, and cannula placement could not account for these individual differences (Fig. S1C). We examined the time course of context + cue-induced reinstatement, and both groups showed robust within session extinction (Fig. 4b; a two-way ANOVA revealed no effect of treatment $F_{(1,16)} = 1.31$, $P = 0.269$, an effect of time $F_{(7,112)} = 9.36$, $P < 0.001$ and a near significant interaction $F_{(7,112)} = 2.03$, $P = 0.057$). However, when each time point was compared to $t = 15$ minutes within treatment groups using a Sidak's multiple comparison test, vehicle-infused rats showed a significant decrease from initial active lever pressing level at $t = 75$ minutes, while BDNF-infused rats decreased at $t = 45$ minutes. Each asterisk shows significant difference of each time point to $t = 15$ minutes within treatments. These results suggest BDNF-infusion potentiates within session extinction during context + cue-induced cocaine seeking in a less robust, but similar manner as during cue-induced reinstatement after extinction training (Fig. 2b).

BDNF acute effect is maintained up to 3 days after microinjection

Numerous studies show long lasting effects of BDNF through TrkB signaling via activation of transcriptional regulators (Ruiz, Shi, & Meffert 2014). Therefore, in a subgroup of animals shown in Figure 2, we examined possible long-lasting effects of BDNF by re-testing rats acutely microinjected with BDNF or vehicle 72 hours after the first reinstatement test. Rats were extinguished for 3 days after the first reinstatement session, and for the second reinstatement test all rats received a vehicle microinjection (Fig. 5a). Rats that previously received a BDNF microinjection showed significantly lower reinstated active lever pressing than rats microinjected with vehicle in the first session (Fig. 5b; a two-way repeated measures ANOVA for active lever presses revealed an effect of extinction/cue $F_{(1,24)} = 33.66$, $P < 0.001$, treatment $F_{(1,24)} = 9.71$, $P = 0.005$ and interaction $F_{(1,24)} = 9.33$, $P = 0.006$). A Sidak's post hoc comparison established a significant potentiation of active lever pressing in presence of cues compared to extinction in the group previously injected with vehicle, but not in the group previously injected with BDNF. The post hoc test also showed a significant decrease of active lever pressing in the presence of cues in the BDNF-microinjected group compared to vehicle control. A two-way ANOVA for inactive lever pressing (Fig. S6) revealed significant treatment factor $F_{(1,24)} = 4.83$, $P = 0.038$, but no effect of extinction/cue factor $F_{(1,24)} = 0.17$, $P = 0.68$ or interaction $F_{(1,24)} = 1.2$, $P = 0.28$. These results suggest that in addition to the acute, non-transcriptional effect of BDNF

through the TrkB signaling cascade, BDNF microinjections into the NAc core induce an enduring reduction in reinstated cocaine seeking. The time course of cue-induced active lever pressing on day 29 (Fig. 5c) was consistent with the total lever pressing data; a two-way ANOVA with repeated measures over time for active lever revealed effects of treatment $F_{(1,24)} = 8.06$, $P = 0.009$ and time $F_{(7,168)} = 9.87$, $P < 0.0001$ but no significant interaction $F_{(7,168)} = 1.36$, $P = 0.225$.

Enduring inhibition by BDNF requires cue-memory reactivation

We next sought to determine if the enduring BDNF-induced inhibition of reinstated cocaine seeking required simultaneous stimulation of TrkB and activation of cue-induced plasticity in the NAc core. To test this, BDNF was microinjected into NAc core before an extinction session (in absence of cues) or in the home cage (in absence of cues and context) (Fig. 6a). An unpaired Student's *t* test revealed that rats infused with BDNF in the NAc core prior to an extinction session showed no difference in active or inactive lever pressing compared with vehicle treatment the day of the extinction session (Fig. 6b; unpaired Student's *t*(10) = 0.99, $P = 0.347$, Fig. S7A; *t*(10) = 1.23, $P = 0.247$). BDNF infusions before an extinction session 3 days prior to cued reinstatement session did not affect lever pressing (Fig. 6b; a two-way repeated measures ANOVA for active lever presses established an effect of extinction/cue $F_{(1,10)} = 70.06$, $P < 0.001$, and no effect of treatment $F_{(1,10)} = 0.37$, $P = 0.556$ or interaction $F_{(1,10)} = 0.01$, $P = 0.977$. A two-way ANOVA revealed no effect by BDNF on inactive lever pressing (Fig. S7A, extinction/cue $F_{(1,10)} = 1.49$, $P = 0.251$, treatment $F_{(1,10)} = 0.99$, $P = 0.344$ and interaction $F_{(1,10)} = 2.36$, $P = 0.156$). The time course of cue-induced active lever pressing on day 29 (Fig. 6c) showed no difference between vehicle and BDNF treatments; a two-way ANOVA with repeated measures over time for active lever revealed a significant effect of time $F_{(7,70)} = 5.41$, $P < 0.0001$ and no significant effects of treatment $F_{(1,10)} = 0.13$, $P = 0.721$ or interaction $F_{(7,70)} = 1.6$, $P = 0.149$.

Animals microinjected with BDNF and returned to the home cage had the same level of drug seeking as rats who received vehicle injections when tested for cue-induced reinstatement 3 days after the microinjection (Fig. 6d; a two-way ANOVA with repeated measures for active lever presses revealed an effect of extinction/cue $F_{(1,14)} = 22.35$, $P = 0.0003$, and no effect of treatment $F_{(1,14)} = 1.50$, $P = 0.241$ or interaction $F_{(1,14)} = 1.75$, $P = 0.207$. A two-way ANOVA revealed no effect by BDNF on inactive lever pressing (Fig. S7B, extinction/cue $F_{(1,14)} = 0.636$, $P = 0.438$, treatment $F_{(1,14)} = 0.01$, $P = 0.929$ and interaction $F_{(1,14)} = 0.0$, $P > 1$). Similarly, no difference between vehicle and BDNF treatments was observed in the time course of cue-induced active lever pressing on day 29 (Fig. 6e); a two-way ANOVA with repeated measures over time for active lever revealed no significant effect of treatment $F_{(1,14)} = 1.71$, $P = 0.213$, time $F_{(7,98)} = 1.65$, $P = 0.132$ or interaction $F_{(7,98)} = 0.58$, $P = 0.771$.

DISCUSSION

We show that endogenous BDNF in the NAc core plays an inhibitory role during drug-free cue-induced cocaine seeking. Acute microinjection of exogenous BDNF into NAc core recapitulated the inhibition of cocaine seeking produced by endogenous BDNF. Both

endogenous and exogenous BDNF effects were mediated by activation of the TrkB signaling. BDNF-induced decrease in cocaine seeking appeared within 15 minutes following intra-NAcore microinjection and was specific to cocaine seeking. Accordingly, no effect by BDNF was observed on inactive lever pressing, spontaneous or cocaine-induced locomotion, or on cue-induced sucrose seeking. BDNF infusions also potentiated within reinstatement session extinction of cocaine seeking after forced abstinence. In accordance with its well-established transcriptional effects, we found BDNF to have lasting effects and the inhibition of cue-induced reinstatement endured for at least 3 days post-infusion. Finally, enduring BDNF inhibition required administration during cue memory retrieval, since BDNF infusions before an extinction session or in the home cage had no effect on subsequent cue-induced reinstatement of cocaine seeking.

BDNF in the core (NAcore) and the shell (NAshell) subregions of the nucleus accumbens

Numerous studies (for review see (Li *et al.*, 2015)) demonstrate that cocaine-induced BDNF alterations vary considerably depending on whether studies were conducted in the NAcore and NAshell subregions of the NAcc. For example, attenuation of BDNF–TrkB signaling in the NAcore increased cue-induced cocaine seeking only in early withdrawal (24 hours), while the same viral manipulation in the NAshell decreased cocaine seeking only in late withdrawal (90 days) (Li *et al.* 2013). Also, following cocaine self-administration, BDNF protein levels and TrkB signaling are increased in the shell, but not in the core (Graham *et al.* 2007). After 45 days of withdrawal, BDNF increase is only measured in the core while at 90 days of withdrawal, BDNF levels are increased in both the core and shell subregions (Li *et al.* 2013). Furthermore, increased BDNF protein expression in the NAcc (including both the core and the shell) induced by an acute intra-PFC exogenous BDNF injection decreases context-, cue- and cocaine-induced cocaine seeking (Berglind *et al.* 2007). Although these experiments are consistent with increases in BDNF levels after cocaine, a recent study showed that repeated non-contingent exposure to cocaine during adolescence decreased BDNF and its signaling in both the NAcore and NAshell (Caffino *et al.* 2018). Additionally, BDNF injections in the NAshell during cocaine self-administration increase cocaine-, cue- and stress-induced reinstatement for up to more than 4 weeks after BDNF treatment (Graham *et al.* 2007). BDNF transmission through TrkB is also necessary for cocaine-induced spine formation in the NAshell (Anderson *et al.* 2017). Similar BDNF injections have not been performed in the NAcore. In our study, we focused on the NAcore and found that BDNF injection minutes before cue-induced reinstatement to cocaine inhibited seeking.

Exogenous BDNF injection and bidirectional transport

BDNF is produced locally in the accumbens (Graham *et al.* 2007) and can undergo retro- and anterograde transport, although retrograde transport seems most prevalent (Zhao *et al.* 2014). Thus, it is likely that a portion of the exogenous BDNF we injected into NAcore traveled to regions both upstream and downstream from the NAcore and that the measured effect on cocaine seeking could be due to a BDNF role in these regions. However, BDNF travel velocity is estimated at 1.06 $\mu\text{m}/\text{sec}$ in dendrites (Kwinter *et al.* 2009; Zhao *et al.* 2014), thus requiring nearly 50 minutes to reach a connected region such as the PFC that is ~ 3.1 mm from the injection site in the rat brain. Since the BDNF injection was microinjected 15 minutes before cue-induced reinstatement, it seems unlikely that biologically active

amounts of the acutely microinjected BDNF reached distal sites creating off-target effects. Concerning the long-lasting effects of previous BDNF microinjections observed in Figure 5, we are unable to exclude a potential role of exogenous BDNF transport to brain regions other than the NAcc.

Transcriptional versus non-transcriptional effects of BDNF

Temporal and spatial specificity are key in the effects of BDNF on cocaine seeking. BDNF-induced activation of TrkB signaling results in receptor dimerization and initiation of a series of phosphorylation events, including phosphorylation of mitogen-activated protein kinase and extracellular signal-regulated kinases pathway (MAPK/ERK), phosphoinositide 3-kinase and protein kinase B (PI3K/Akt) and phospholipase C γ (PLC γ 1) (Reichardt 2006). Some of these signaling events lead to activation of transcription factors such as cyclic AMP response element binding protein (CREB) (Lu *et al.* 2006), which regulates the expression of a wide array of target genes affected by BDNF. This effect of BDNF on gene expression most likely underlies the lasting effects of BDNF observed in the literature, including repeated BDNF infusions in the NAcc during cocaine SA potentiating cocaine intake, as well as context-, cue- and stress-induced cocaine seeking (Graham *et al.* 2007). In the same study, the authors neutralized BDNF by infusing BDNF antibodies in the NAcc during stable cocaine SA and measured a decrease of cocaine intake, as well as reduced footshock-induced reinstatement of cocaine seeking (Graham *et al.* 2007). When BDNF is infused in the PFC after the last cocaine exposure, it decreases context-, cue- and cocaine primed-induced reinstatement to cocaine seeking (Berglind *et al.* 2009). These studies focus on the long lasting, likely transcriptional effects of BDNF, while we focused here on faster mechanisms by administering BDNF or TrkB antagonists only 15 minutes before cued-reinstatement and in the absence of cocaine. We hypothesize that transcriptional effects of BDNF underlie the inhibition of cued-reinstatement observed 3 days post infusion (Fig. 5b); although this could also be due to post-transcriptional changes induced by strong activation of the TrkB signaling and consequent phosphorylation cascade. For instance, BDNF-TrkB activation modulates alcohol-induced neuroadaptations by altering miRNA expression in the PFC (Heilig *et al.* 2017).

Role of context in the actions of BDNF

BDNF inhibits cue-induced cocaine seeking when it is infused minutes before cued-reinstatement. When infused before an extinction session (with presentation of the context but in the absence of cues, Fig. 6b) or in the home cage (Fig. 6d) BDNF had no effect on cocaine seeking. These results suggest that BDNF-induced inhibition of drug seeking requires a time window during which cue-induced reactivation of the neural circuit mediating seeking is paired with simultaneous TrkB signaling activation. Memory reactivation converts an inactive memory to a malleable state that can be reconsolidated (Gisquet-Verrier & Riccio 2012). Since BDNF decreases cue-induced cocaine seeking, we hypothesize that BDNF-induced activation of TrkB signaling weakens the memory of the cue associated to the drug during SA training. This would result in weaker activation of the circuit inducing seeking and thereby decrease cocaine seeking, however, the precise mechanism remains to be determined.

Role of D1- versus D2-MSNs

TrkB receptor gene is expressed by both D1- and D2-MSNs (Freeman *et al.* 2003); although TrkB mRNA expression is greater in D2-compared to D1-MSNs (Lobo *et al.* 2010). Genetically inhibiting the expression of TrkB specifically in D1-MSNs potentiates cocaine CPP and sensitization compared to control mice, while knocking out TrkB in D2-MSNs decreases CPP and sensitization (Lobo *et al.* 2010). Our study did not dissect the role of D1-versus D2-MSNs, it is however generally understood that activation of the D1 pathway promotes reward seeking, while D2 pathway activation can lead to aversion or reduced seeking (Kravitz, Tye, & Kreitzer 2012; Calipari *et al.* 2016). We hypothesize that BDNF could selectively activate D2-MSNs, potentiate within trial extinction and decrease cocaine seeking. Supporting this hypothesis, infusions of BDNF or systemic injections of a synthetic TrkB receptor agonist 7,8, dihydroxyflavone, enhance extinction of cocaine CPP (Otis, Fitzgerald, & Mueller 2014).

CONCLUSIONS

We investigated the effect of endogenous BDNF secretion on cocaine seeking in the NAc and discovered that BDNF-induced TrkB signaling during cued-reinstatement promoted within session extinction and decreased cocaine seeking. We were able to pharmacologically recreate the inhibition of cocaine seeking by acutely microinfusing BDNF in the NAc before cue-induced reinstatement. This effect was specific to cocaine seeking, and did not alter inactive lever pressing, spontaneous or cocaine-induced locomotion, or sucrose seeking. The inhibitory effect of BDNF transmission induced during cued cocaine seeking contrasts with some other studies examining effects of chronic or constitutive changes in BDNF levels and may indicate a distinction between non-translational effects of acute BDNF transmission and enduring effects mediated by changes in gene expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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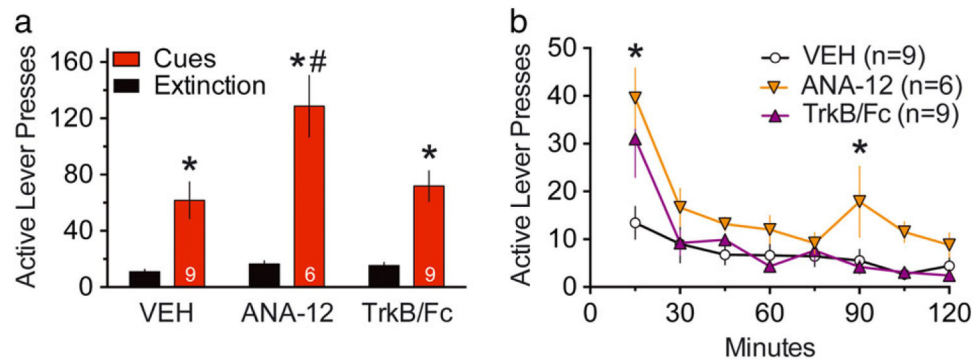


Figure 1. Blockade of endogenous BDNF-induced TrkB signaling in the NAc core potentiated cue-induced cocaine seeking.

(a) Active lever pressing during 2-hour cued-reinstatement test, all drugs were administered into NAc core 15 minutes prior to initiating the session, including vehicle (VEH; 0.5 μ l/side), ANA-12 (0.1 pg/0.5 μ l/side) and TrkB/Fc (650 ng/0.5 μ l/side). The number of animals is indicated in the columns. A two-way ANOVA for active lever revealed effects of extinction/reinstatement $F_{(1,21)} = 77.12$, $P < 0.0001$, treatment $F_{(2,21)} = 5.57$, $P = 0.012$ and an interaction $F_{(2,21)} = 4.86$, $P = 0.019$. * $P < 0.05$ comparing extinction to reinstatement for all groups. # $P < 0.05$ comparing reinstatement of ANA-12 to VEH, Sidak's multiple comparison test. (b) Time course of active lever pressing during 2-hour cued-reinstatement for VEH, ANA-12 and TrkB/Fc microinjected groups. A two-way ANOVA with repeated measures over time for active lever revealed effects of treatment $F_{(2,21)} = 5.86$, $P = 0.001$, time $F_{(7,147)} = 19.50$, $P < 0.0001$ and interaction $F_{(14,147)} = 2.33$, $P = 0.006$. * $P < 0.05$ comparing ANA-12 or TrkB/Fc to VEH group at each time point, Sidak's multiple comparison test

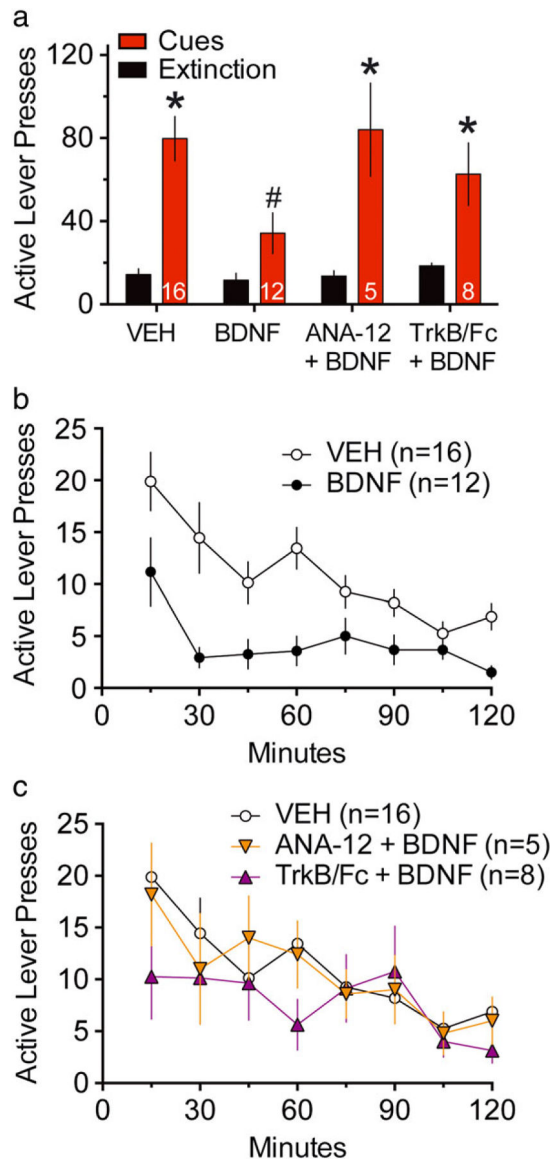


Figure 2. Acute exogenous BDNF infusion into NAc core potentiated within trial extinction and inhibited cue-induced cocaine seeking through TrkB.

(a) Active lever pressing during 2 h cued-reinstatement test, vehicle VEH (0.5 μ l/side), BDNF (1 μ g/0.5 μ l/side), BDNF (1 μ g/0.5 μ l/side) + ANA-12 (0.1 μ g/0.5 μ l/side) or BDNF (1 μ g/0.5 μ l/side) + TrkB/Fc (650 ng/0.5 μ l/side) were administered into NAc core 15 minutes prior to initiating the session. A two-way ANOVA for active lever presses revealed effects of extinction/reinstatement $F_{(3,37)} = 55.54$, $P < 0.0001$, treatment $F_{(3,37)} = 3.04$, $P = 0.041$ and interaction $F_{(3,37)} = 3.21$, $P = 0.034$. * $P < 0.05$ comparing extinction to reinstatement within groups. # $P < 0.05$ comparing BDNF to VEH or ANA-12 + BDNF during cued-reinstatement, Sidak's multiple comparison test. (b) Time course of active lever pressing during 2-hour cued-reinstatement for VEH and BDNF microinjected groups. Two-way ANOVA for active lever revealed effects of time $F_{(7,259)} = 8.91$, $P < 0.0001$, treatment $F_{(3,37)} = 4.34$, $P = 0.010$ but no significant interaction $F_{(21,259)} = 1.29$, $P = 0.181$. (c) Time course of active lever pressing during 2-hour cued-reinstatement for VEH, ANA-12 + BDNF and

TrkB/Fc + BDNF microinjected groups. The vehicle group is the same as in b, and the four treatment groups were divided between panels b and c for illustrative clarity. For statistics, see ANOVA results in b

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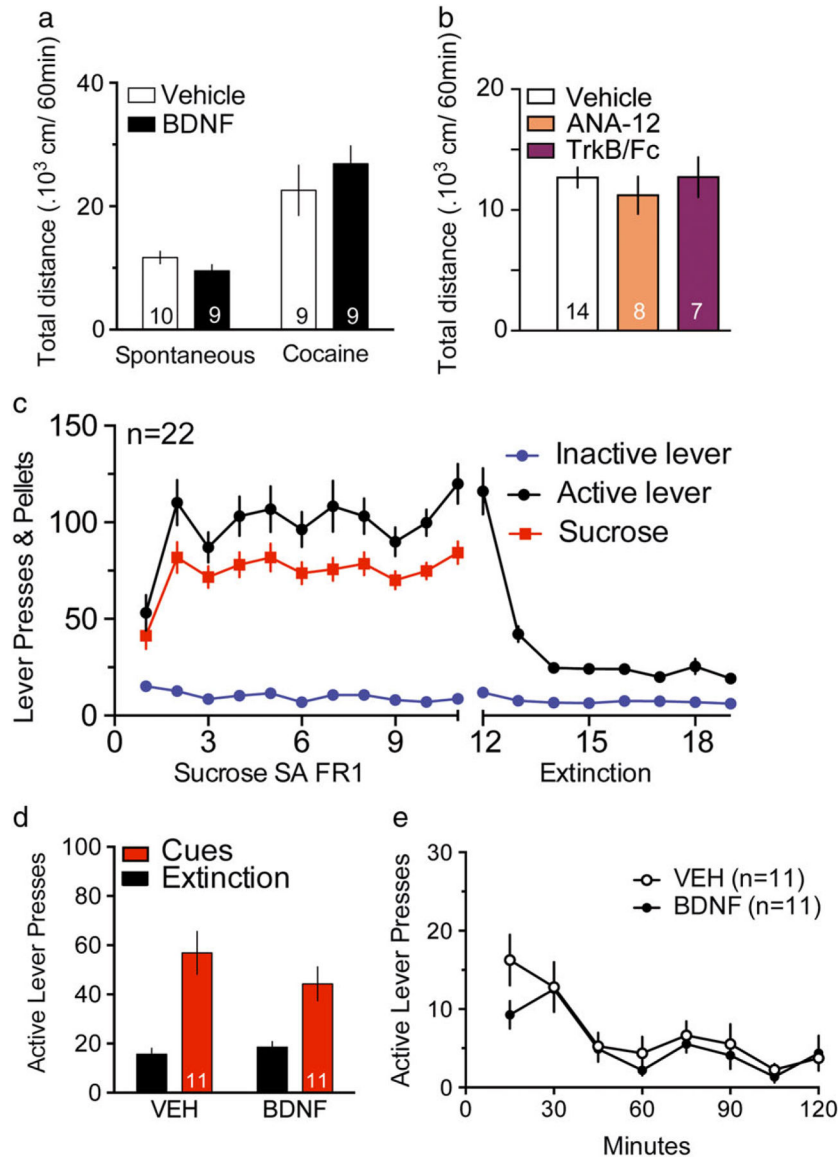


Figure 3. Acute exogenous BDNF infusion in the NAc core was specific to cue-induced cocaine seeking behavior and had no effect on spontaneous or cocaine-induced locomotion or sucrose seeking.

(a) 1-hour spontaneous locomotion in an unadapted open field was measured 15 minutes after VEH (0.5 μ l/side) or BDNF (1 μ g/0.5 μ l/side) injections in the NAc core. An unpaired Student's test revealed no differences between groups, $t(17) = 1.53$, $P = 0.140$. Cocaine-induced locomotion was measured immediately after cocaine injection (20 mg/kg, i.p.) in an adapted open field, 15 minutes after VEH (0.5 μ l/side) or BDNF (1 μ g/0.5 μ l/side) infusions in the NAc core (unpaired Student's test $t(16) = 0.86$, $P = 0.40$). (b) 1-hour spontaneous locomotion in an unadapted open field was measured 15 minutes after VEH (0.5 μ l/side), ANA-12 (0.1 pg/0.5 μ l/side) or TrkB/Fc (650 ng/0.5 μ l/side) injections in the NAc core. A one-way ANOVA revealed no differences between groups $F(2,26) = 0.43$, $P = 0.65$. (c) Rats sucrose SA acquisition with fixed ratio 1 (FR1) and extinction training. (d) Vehicle VEH (0.5 μ l/side) or BDNF (1 μ g/0.5 μ l/side) were administered into NAc core 15 minutes prior to

initiating 2-hour cued reinstatement. The number of animals per group is located at the bottom of columns. A two-way repeated measures ANOVA for active lever presses revealed an effect of extinction/cue $F_{(1,20)} = 38.80$, $P < 0.0001$, but no effect of treatment $F_{(1,20)} = 0.58$, $P = 0.456$ or interaction $F_{(1,20)} = 2.01$, $P = 0.171$. (e) Time course of active lever pressing during 2-hour cued-reinstatement for sucrose for VEH and BDNF microinjected groups. A two-way repeated measures ANOVA for active lever presses revealed an effect of time $F_{(7,140)} = 11.07$, $P < 0.001$, but no significant effect of treatment $F_{(1,20)} = 1.3$, $P = 0.269$ or interaction $F_{(7,140)} = 0.88$, $P = 0.521$

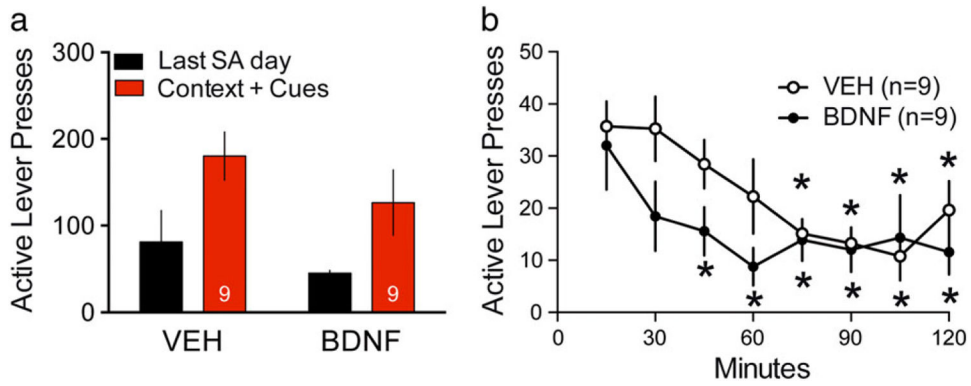


Figure 4. Acute exogenous BDNF infusion in the NAc core potentiated within trial extinction during context + cue-induced reinstatement after forced abstinence.

(a) Active lever pressing during 2-hour context + cue-reinstatement test after forced abstinence, all drugs were administered into NAc core 15 minutes prior to initiating the session, including vehicle VEH (0.5 μ l/side) and BDNF (1 μ g/0.5 μ l/side). Two-way repeated measures ANOVA for active lever presses revealed an effect of last day of SA versus context + cue-induced reinstatement $F_{(1,16)} = 8.12$, $P = 0.012$, but no effect of treatment $F_{(1,16)} = 2.62$, $P = 0.125$ or interaction $F_{(1,16)} = 0.08$, $P = 0.783$. (b) Time course of active lever pressing during 2-hour context + cue-reinstatement test after forced abstinence for VEH and BDNF microinjected groups. A two-way ANOVA revealed no effect of treatment $F_{(1,16)} = 1.31$, $P = 0.269$, an effect of time $F_{(7,112)} = 9.36$, $P < 0.001$ and a near significant interaction $F_{(7,112)} = 2.03$, $P = 0.057$. * $P < 0.05$ comparing active lever pressing of each time point to $t = 15$ minutes within treatments, Sidak's multiple comparison test

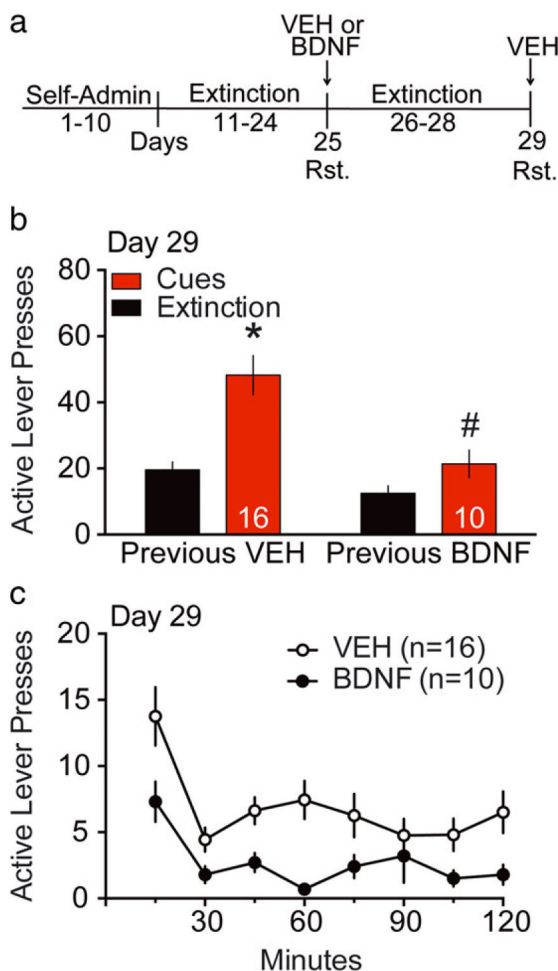


Figure 5. BDNF inhibition on cocaine seeking was sustained for at least 3 days post infusion. (a) Experiment timeline. Rats underwent at least 10 days of cocaine SA (Self-Admin), followed by at least 10 days of extinction training. Vehicle (VEH) or BDNF were microinjected into NAcCore 15 minutes before the first cued-reinstatement (Rst, Day 25), while all animals received vehicle injections before the second cued-reinstatement (Rst, Day 29). (b) Active lever pressing during the second 2-hour cued-reinstatement test. Rats were microinjected VEH (0.5 μ l/side) or BDNF (1 μ g/0.5 μ l/side) into the NAcCore 15 minutes before the first cued-reinstatement. The second reinstatement presented here occurred 3 days after the first. Each animal received a maximum of two reinstatement trials. Number at the bottom of columns represents the N for each treatment group. A two-way repeated measures ANOVA for active lever presses revealed an effect of extinction/cue $F_{(1,24)} = 33.66$, $P < 0.001$, treatment $F_{(1,24)} = 9.71$, $P = 0.005$ and interaction $F_{(1,24)} = 9.33$, $P = 0.006$. * $P < 0.05$ comparing extinction to cued-reinstatement within groups, # $P < 0.05$ comparing cued-reinstatement between VEH and BDNF, Sidak's multiple comparison test. (c) Time course of active lever pressing during 2-hour cue-reinstatement test (Day 29) for VEH and BDNF microinjected groups. A two-way ANOVA with repeated measures over time for active lever revealed effects of treatment $F_{(1,24)} = 8.06$, $P = 0.009$ and time $F_{(7,168)} = 9.87$, $P < 0.0001$ but no significant interaction $F_{(7,168)} = 1.36$, $P = 0.225$

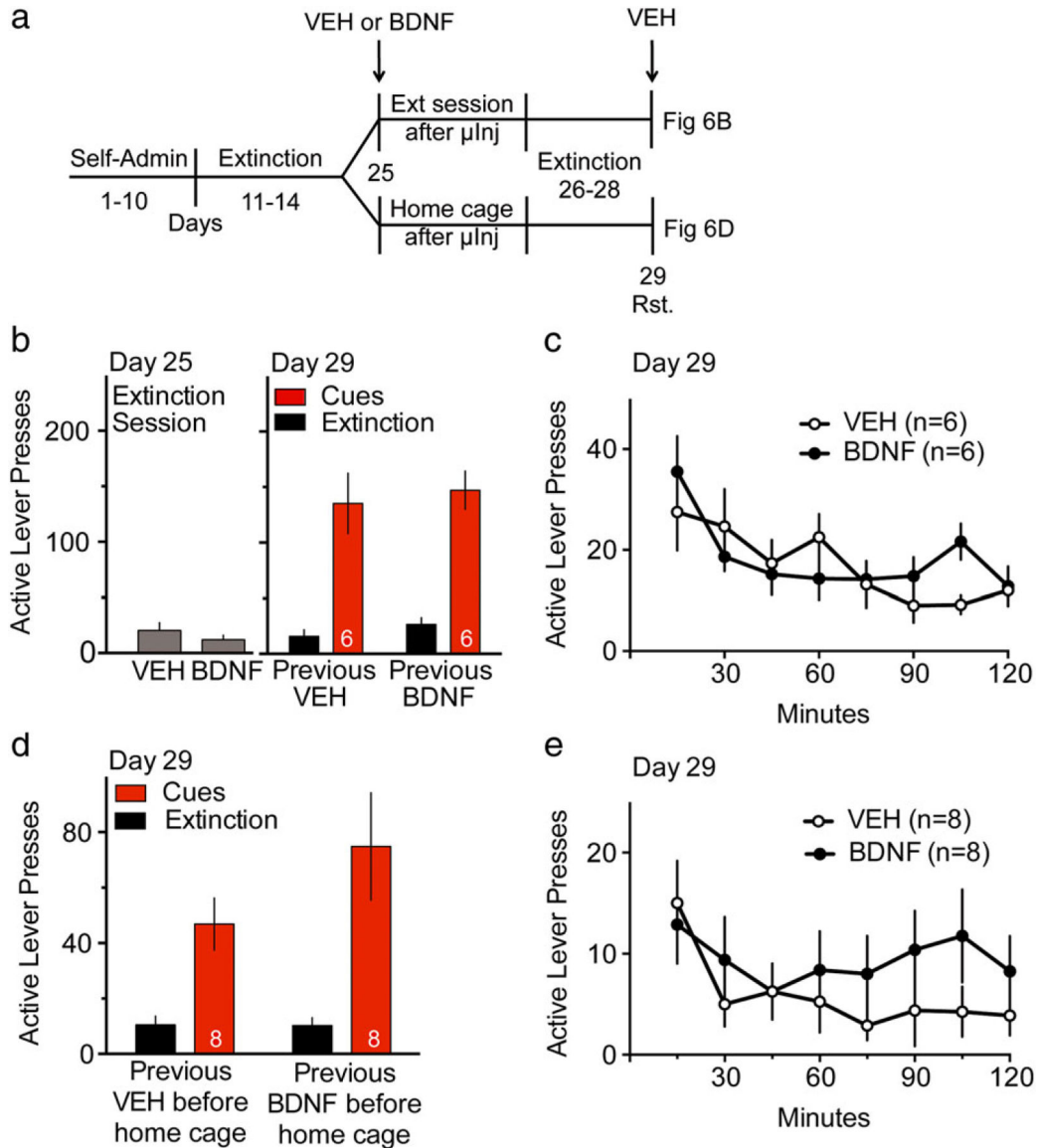


Figure 6. BDNF inhibition on cocaine seeking required cue-memory presentation.

(a) Experiment timeline. Rats underwent at least 10 days of cocaine SA (Self-Admin), followed by at least 10 days of extinction training. Vehicle (VEH) or BDNF were microinjected into NAcCore 15 minutes before an extinction (Ext) session or before returning to the home cage at Day 25, while all animals received vehicle injections before the second cued-reinstatement (Rst, Day 29). (b) Active lever pressing during the extinction session preceded by VEH (0.5 μ l/side) or BDNF (1 μ g/0.5 μ l/side) infusions into the NAcCore and during the first 2-hour cued-reinstatement test 3 days later. An unpaired Student's $t(10) = 0.99$, $P = 0.347$ shows no difference between vehicle or BDNF microinjection on active lever pressing during extinction session Day 25. On day 29, a two-way repeated measures ANOVA for active lever presses revealed an effect of extinction/cue $F_{(1,10)} = 70.06$, $P < 0.001$, and no effect of treatment $F_{(1,10)} = 0.37$, $P = 0.556$ or interaction $F_{(1,10)} < 0.01$, $P = 0.977$. (c) Time course of active lever pressing during 2-hour cue-reinstatement test (Day 29)

for groups previously microinjected with VEH or BDNF in the NAc core before an extinction session. A two-way ANOVA with repeated measures over time for active lever revealed a significant effect of time $F_{(7,70)} = 5.41$, $P < 0.0001$ and no significant effects of treatment $F_{(1,10)} = 0.13$, $P = 0.721$ or interaction $F_{(7,70)} = 1.6$, $P = 0.149$. (d) Active lever pressing during the first 2-hour cued-reinstatement test. Three days prior to cued-reinstatement, rats were microinjected VEH (0.5 μ l/side) or BDNF (1 μ g/0.5 μ l/side) into the NAc core and placed back in their home cage, with no recording of lever pressing activity in the home cage. A two-way repeated measures ANOVA for active lever presses revealed an effect of extinction/cue $F_{(1,14)} = 22.35$, $P = 0.0003$, and no effect of treatment $F_{(1,14)} = 1.50$, $P = 0.241$ or interaction $F_{(1,14)} = 1.75$, $P = 0.207$. The number of animals per group is located at the bottom of columns. (e) Time course of active lever pressing during 2-hour cue-reinstatement test (Day 29) for groups previously microinjected with VEH or BDNF in the NAc core prior to return to home cage. A two-way ANOVA with repeated measures over time for active lever revealed no significant effect of treatment $F_{(1,14)} = 1.71$, $P = 0.213$, time $F_{(7,98)} = 1.65$, $P = 0.132$ or interaction $F_{(7,98)} = 0.58$, $P = 0.771$