Supplementary Methods

Cocaine self-administration procedures in rats. To facilitate subsequent acquisition of cocaine self-administration, animals initially were maintained at 85% original body weight and trained to press a lever for 45 mg sucrose pellets in operant chambers (Med Associates, Georgia, VT) on a fixed ratio 1 (FR1) reinforcement schedule until acquisition criteria were achieved (100 pellets self-administered for 3 consecutive days). Animals were then fed *ad libitum* for at least 1 day prior to surgical brain cannulation and/or intravenous catheterization as described previously. Bilateral 26 gauge guide cannulae (Plastics One, Roanoke, VA, USA) were implanted in the NAc shell (1.7 mm anterior to bregma, ±0.8 mm lateral, and −5.7 mm ventral to dura) or caudate-putamen (1.7 mm anterior to bregma, ±1.5 mm lateral, and −3.7 mm ventral to dura) with level skull according to Paxinos and Watson. Treatments were delivered through 30 gauge bilateral infusion cannulae cut to extend 1 mm beyond guide cannulae.

Following a 1 week recovery period, animals were trained to self-administer intravenous injections of cocaine hydrochloride (500 µg per kg per 50 µl injection) delivered over 2.5 s (FR1) in daily 4-h sessions as described above. Each injection was accompanied by illumination of a cue light above the active lever, and followed by an additional 12.5 s time-out period (TO) when the cue and house lights were extinguished and lever press responses had no programmed consequence. For behavioral experiments, the response requirement was gradually increased to 5 lever presses per injection (FR5) and training continued until cocaine intake stabilized (number of total infusions varied <10% from the mean of three consecutive sessions). Following acquisition and stabilization (3–4 weeks), animals received a bilateral NAc shell or caudate-putamen infusion of BDNF (2.5 µg per side), anti-BDNF (5.0 µg per side, azide free, Cat # AB1513P, Chemicon, Temecula, CA), IgG (5.0 µg per side) or the PBS vehicle in 0.5 µl over 3 min immediately after the termination of 5 consecutive daily self-administration sessions. Three days after the last infusion, animals were subjected to a between-session dose-response test with
each of 5 injection doses (0, 30, 100, 300, 1000 g kg⁻¹) available in descending order in 4-h test sessions. Catheter patency was verified after testing by brief anesthesia with sodium methohexital (0.1 mg in 0.1 ml).

In a separate experiment, animals were trained to self-administer cocaine, stabilized on a FR5 reinforcement schedule, and given 5 repeated intra-NAc shell infusions as described above, prior to testing on a progressive ratio reinforcement schedule. Three days after the last intra-NAc infusion, animals self-administered 1 of 2 injection doses of cocaine (250 or 750 μg per kg per injection), where the response requirement for each successive injection increased by progressive increments according to the following series: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, etc. according to response requirement = [5e(injection # x 0.2)] – 5.³ Each dose was tested in two consecutive daily sessions in counterbalanced dose order over 4 test sessions. The highest ratio of responses/injection completed before a 1-h period when no additional injections were earned was analyzed using data from the second test at each dose.

**Extinction/reinstatement of cocaine seeking in rats.** Animals that received NAc shell or caudate-putamen infusions before fixed ratio dose-response testing remained in their home cages for 10 days prior to extinction/reinstatement testing in cocaine withdrawal. Extinction testing was conducted in 5 daily 4-h sessions in the absence of response-contingent cocaine or injection cues, and non-reinforced responding at the drug-paired lever was recorded. During the following week, the ability of specific stimuli to induce cocaine-seeking behavior was assessed in five consecutive reinstatement test sessions as described⁴. Reinstatement sessions were identical to 4-h extinction sessions, except that reinstating stimuli were presented before or during the last hour of the session according to the following sequence of daily tests: cue presentation (every 2 min for 1 h), immediately after an intravenous cocaine priming injection (saline, 500 and 2,000 μg kg⁻¹, iv, in counterbalanced order), and after 30 min of intermittent footshock stress (1.0 mA in 0.5 s
with random intervals averaging 30 s). Non-reinforced responding at both drug-paired and unpaired levers was analyzed for each 1-h reinstatement test. Responding at the unpaired lever failed to increase in any group relative to saline priming (not shown), but footshock stress caused a few animals to respond on both levers.

**Extinction/reinstatement of sucrose seeking in rats.** Bilateral guide cannulae were implanted in the NAc shell of drug-naïve rats, and animals were trained to self-administer sucrose pellets as described above. Following acquisition, the reinforcement schedule was changed to FR5:TO 15 s (maximum of 100 pellets available) to mimic conditions for cocaine self-administration. Animals were trained on this schedule until stable responding was achieved (latency to self-administer 100 pellets varied < 10% of 3 consecutive sessions). Stabilized animals received NAc shell infusions of BDNF, anti-BDNF, or PBS immediately after 5 consecutive daily tests as described above, and 3 days later animals were tested for an additional 5 days of sucrose self-administration. Following 10 days in the home cage, animals were tested in 5 daily 4-h extinction test sessions in the absence of sucrose or cue reinforcement. After extinction testing, reinstatement of sucrose seeking was assessed by non-contingent delivery of sucrose pellets, 2 pellets initially followed by 1 pellet every 2 minutes for a 1-h test session (a total of 32 pellets). Non-reinforced responding on the lever previously associated with sucrose delivery was recorded.

**Verification of infusion sites.** Following successful completion of cocaine or sucrose self-administration experiments, rats were anesthetized with chloral hydrate (400 mg kg⁻¹, i.p.) and given bilateral 0.5 µl cresyl violet infusions through the guide cannulae as described above. Immediately following the infusions, animals were decapitated, brains dissected and 0.8 mm
thick coronal slices through the forebrain were analyzed under a dissecting microscope for location of infusion sites.

**Self-administration procedures in mice.** Male, floxed BDNF mice under ketamine/xylazine anesthesia received bilateral stereotaxic infusions of AAV-GFP or AAV-CreGFP through 33 gauge microsyringes oriented at a 10° angle (1.5 mm anterior to bregma, ±1.5 mm lateral, and 4.4 mm ventral to dura). After 2 weeks, mice were tested for instrumental learning with sucrose pellet reinforcement in daily 1-h sessions following 16 hrs food deprivation. Spontaneous lever-press behavior in the absence of reinforcement was measured in operant test chambers (Med Associates) in the initial 1-h test. On subsequent test days, following 16 h of food deprivation mice were allowed to lever press for 25 mg sucrose pellets (FR3) until they achieved acquisition criteria (25 pellets per session) and continued lever-press training for an additional two test sessions. The number of sessions to meet acquisition criteria and the percent of mice meeting criteria at each day were determined.

Following acquisition of sucrose self-administration, mice fed ad libitum were surgically implanted with a chronic indwelling jugular catheter as described and allowed at least 3 days to recover before cocaine self-administration testing. Acquisition of cocaine self-administration (500 µg per kg per 50 µl injection delivered over 2.5 s concurrent with cue light and 2.9 kHz tone) was tested in daily 1-h sessions on a FR1:TO 8 s reinforcement schedule over 8–10 days. After acquisition testing, the response requirement was gradually increased to FR5 until self-administration stabilized to within 15% of the mean of three consecutive sessions. Mice were subsequently allowed to self-administer descending injection doses of cocaine, each for two consecutive daily 1-h sessions, beginning with 1000 µg per 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. Fifteen of 16 mice entering fixed ratio dose-response testing
maintained catheter patency and were used in the analysis. Catheter patency was verified after each test phase with sodium methohexital (25 µg in 50 µl).

Locomotor testing was conducted in separate study groups of floxed BDNF mice receiving bilateral intra-NAc infusions of AAV-GFP or AAV-CreGFP 14–21 days earlier as described above. Mice were tested for dopamine receptor-mediated locomotor activity in rectangular test chambers equipped with photocell detectors. Animals received two 3-h habituation sessions. Over the next 4 days, mice were given injections of the D1 receptor agonist SKF 81297 (saline, 0.3, 1.0, and 3.0 mg kg⁻¹, sc) after 2 h of further habituation in 3 h test sessions in a counterbalanced fashion. Total horizontal locomotor activity (photobeam counts/min) was recorded by computer data acquisition software (San Diego Instruments) during the third h of the session.

**Immunohistochemical localization of AAV expression.** Following successful completion of sucrose and cocaine self-administration testing (~8 weeks post-AAV), mice were transcardially perfused with 4% para-formaldehyde, brains cryoprotected for 4 days in 30% sucrose, and 35 µm coronal sections were collected from frozen brains for anti-GFP immunolabeling. Sections were dehydrated for 2 days, washed with 1% H2O2 in PBS for 30 min, and blocked with 3% normal donkey serum (NDS) in PBS containing 0.3% Tween20 for 1 hr at 23° C. Mounted sections were incubated overnight with rabbit primary anti-GFP (1:3000, Molecular Probes, Carlsbad, CA, USA), followed by 90 min in biotinylated donkey anti-rabbit IgG (1:200; DAKO Corporation, Carpinteria, CA, USA) secondary in 1.5% NDS/PBS and amplified by avidin-conjugated peroxidase (Vector Laboratories, Burlingame, CA) CY2-tyramide signal amplification system (Perkin-Elmer, Norton, Ohio) followed by Nissl counterstaining.
Supplementary Material References


