Cell Type Specific Loss of BDNF Signaling Mimics Optogenetic Control of Cocaine Reward

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Supporting On-Line Material:

Supplemental Figures
Supplemental Methods
Supplemental References

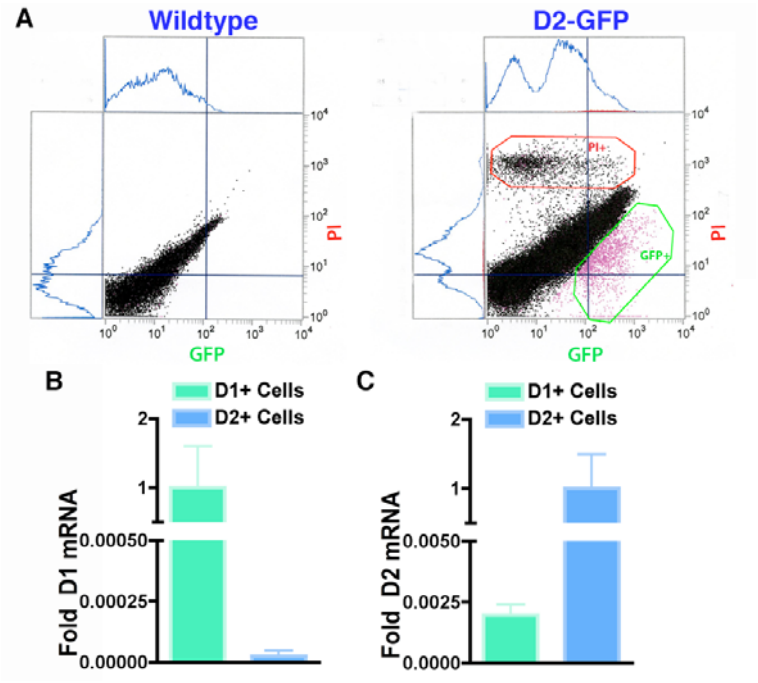

Figure S1. FACS purification of D1+ and D2+ MSNs from whole striatum for gene expression profiling. (**A**) Plots for fluorescence activated cell sorting (FACS) from wildtype (no GFP expression) and D2-GFP mice. Wildtype controls were used to gate GFP signal (x-axis) and propidium iodide signal (PI, y-axis). D2-GFP plot shows a pure population of GFP+ neurons that were sorted for gene expression analyses. PI was used to gate out dead cells. D1+ MSNs from D1-GFP mice were similarly sorted with equivalent results (data not shown). (**B,C**) Purified D1+ cells displayed enrichment of D1 receptor mRNA but very little expression of D2 receptor mRNA. In contrast, D2+ cells exhibited enrichment of D2 receptor mRNA and minimal expression of D1 receptor RNA. For detailed protocols on this methodology refer to published studies (*S1*).

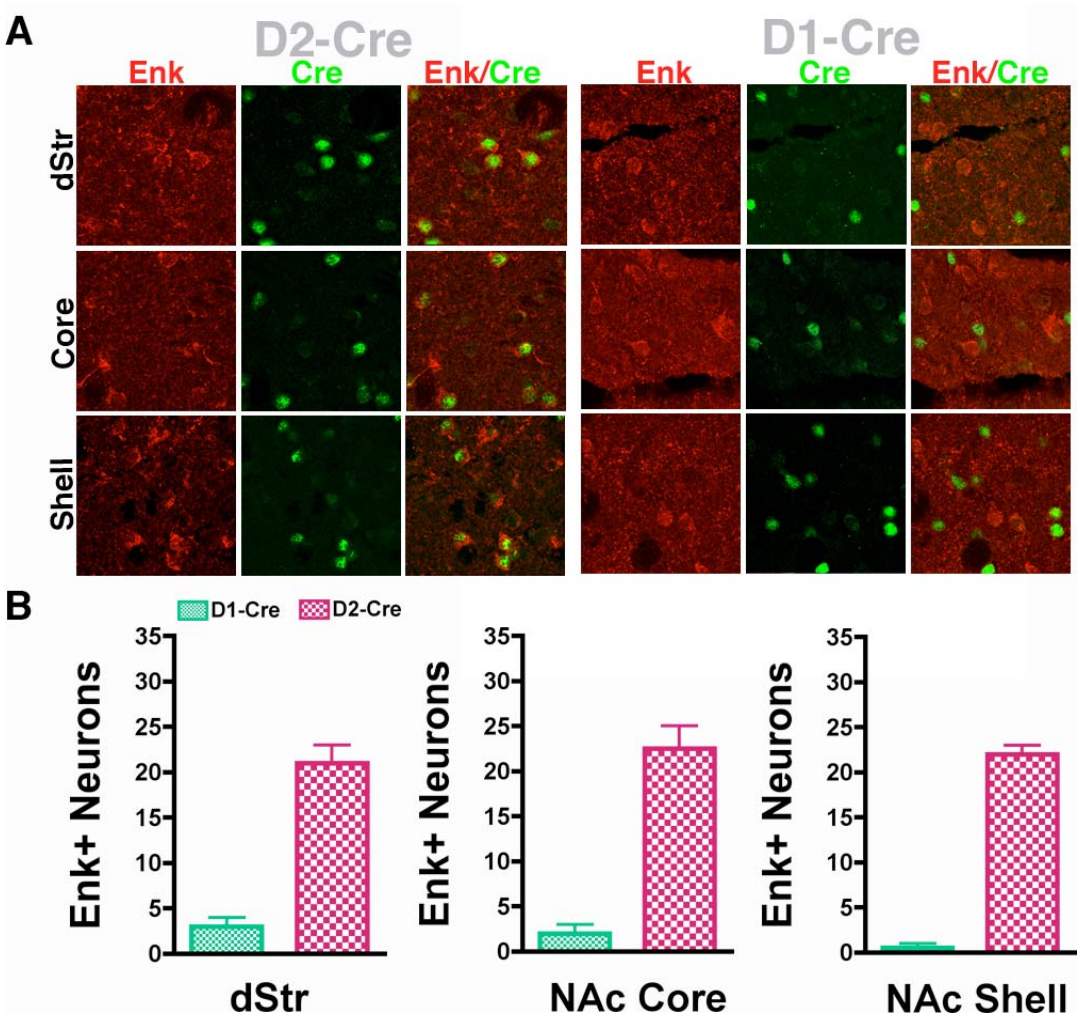

Fig S2. Enkephalin immunostaining confirms selectivity of Cre expression in D1-Cre and D2-Cre mice. (A) Brain sections of D1-Cre and D2-Cre mice containing dorsal striatum (dStr) and nucleus accumbens (NAc-Core and Shell) were double immunostained for enkephalin (Enk, red) and Cre (green). Minimal colocalization was observed between Cre and Enk in D1-Cre sections, whereas very high colocalization was observed in D2-Cre sections. **(B)** Quantification of Enk/Cre colocalization reveals significantly higher colocalization in D2-Cre dStr and NAc core and shell. Student's t-test, *p <0.01.

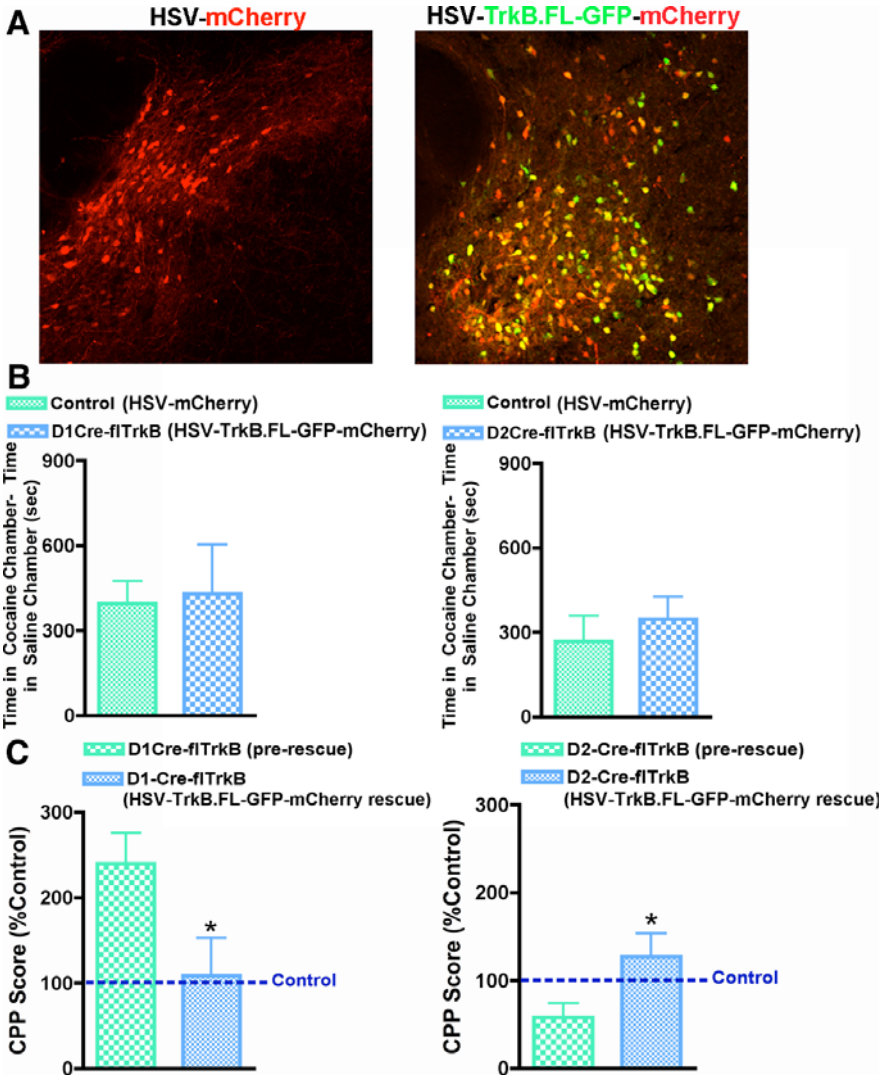

Fig S3. TrkB overexpression in the NAc of D1-Cre-flTrkB and D2-Cre-flTrkB mice rescues the cocaine CPP phenotypes. (A) Expression of HSV-mCherry in the NAc of control mice and HSV-TrkB-GFP-mCherry (*S2*) in the NAc of D1-Cre-flTrkB and D2-Cre-flTrkB mice. **(B)** D1-Cre-flTrkB and D2-Cre-flTrkB mice expressing HSV-TrkB-GFP-mCherry in the NAc display no significant change in cocaine (7.5 mg/kg) CPP relative to controls expressing HSV-mCherry in the NAc. n = 10 per group **(C)** Both D1-Cre-flTrkB and D2-Cre-flTrkB rescue groups were normalized to controls to compare to pre-rescue CPP results in each group also normalized to controls. A significant decrease in cocaine CPP is observed in the D1-Cre-flTrkB rescue group compared to the pre-rescue group. In contrast, a significant increase is observed in the D2-Cre-flTrkB rescue group compared to the pre-rescue group. Student's t-test, *$p < 0.05$.

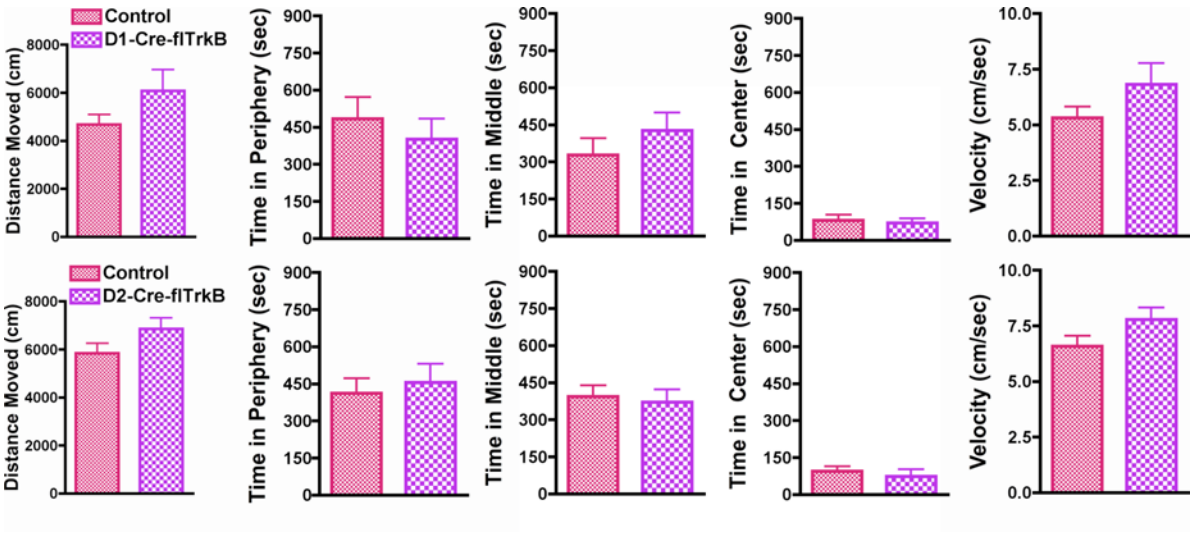

Fig S4. D1-Cre-flTrkB and D2-Cre-flTrkB mice display normal baseline behaviors in a novel environment. No significant differences were found in locomotor activity, time in open field arena regions, and velocity in a novel environment in D1-Cre-flTrkB and D2-Cre-flTrkB mice compared to controls.

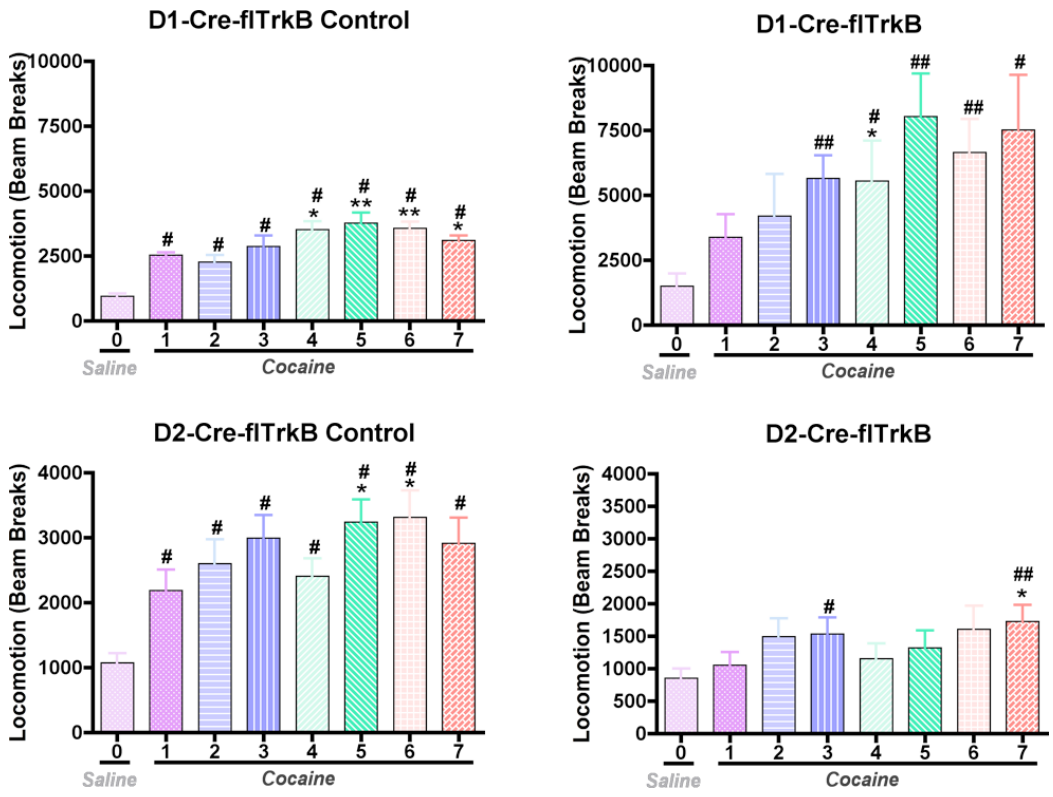

Fig S5. Cocaine (10 mg/kg) locomotor sensitization in D1-Cre-flTrkB, D2-Cre-flTrkB, and control mice. Repeated measures ANOVA, $p < 0.01$ for all groups. Student t-test post hoc analysis, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ compared to saline day 0, $^{**}p < 0.01$ and $^{*}p < 0.05$ compared to cocaine day 1.

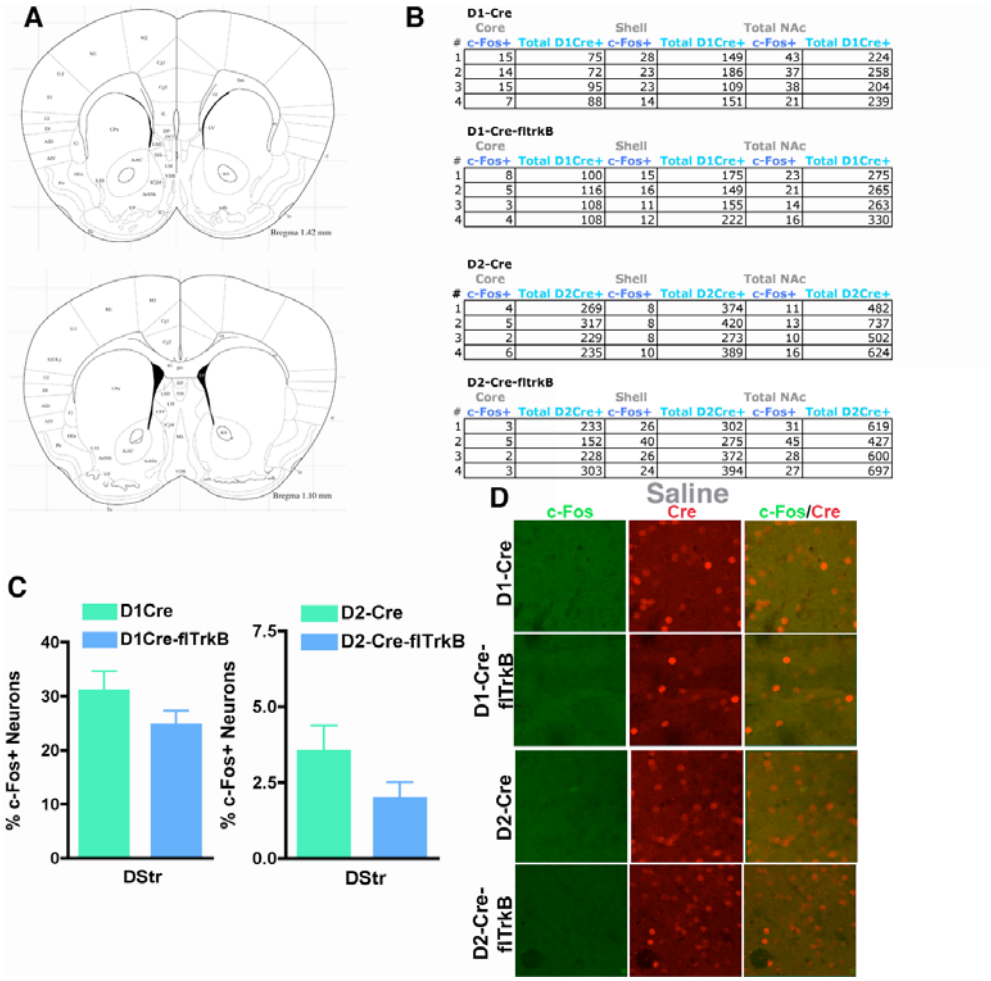

Fig S6. c-Fos expression in D1-Cre-flTrkB and D2-Cre-flTrkB mice after cocaine and saline exposure. **(A)** Diagram of Bregma regions in which we counted c-Fos+ cells in D1-Cre-flTrkB and D2-Cre-flTrkB mice and their controls. Neurons were counted from random sampling of 135 μm x 135 μm scanned confocal images between Bregma 1.5-1.0 from both hemispheres of ~3-4 brain sections with a thickness of 35 μm. **(B)** A table with the raw values of neurons counted. Approximately >200 and >400 Cre positive neurons were counted in the NAc of D1-Cre-flTrkB and D1-Cre controls and D2-Cre-flTrkB and D2-Cre controls, respectively. The number of c-Fos positive neurons were then counted within those groups. **(C)** No change in c-Fos levels was observed in dorsal striatum (dStr) in both groups. **(D)** Minimal to no c-Fos reactivity was observed in saline treated mice.

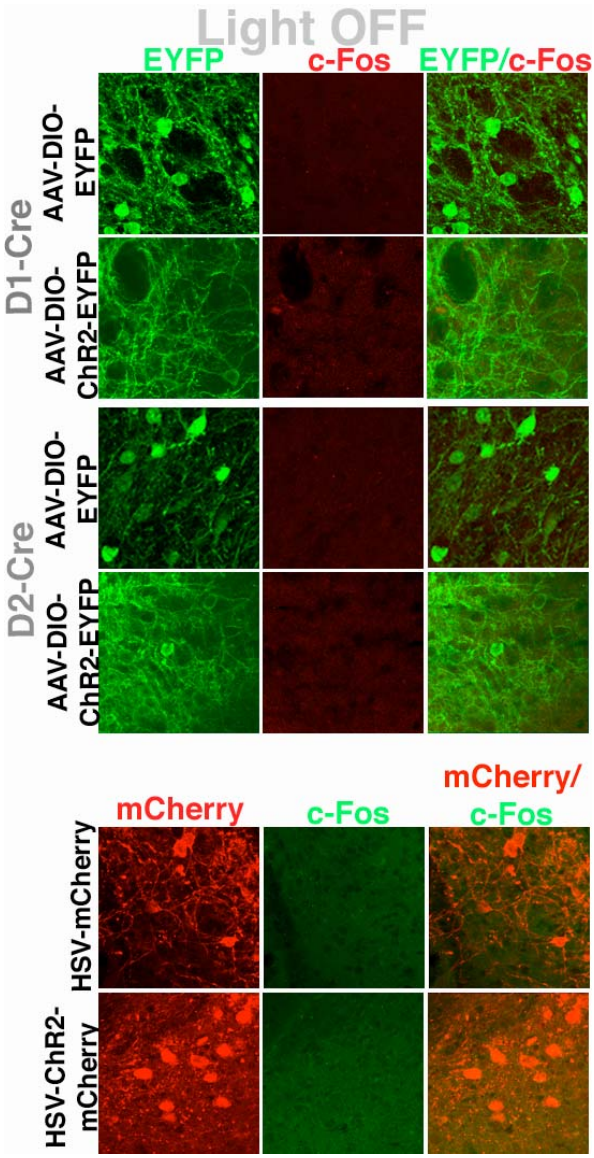

Fig S7. Minimal to no c-Fos expression is observed in ChR2 and EYFP/mCherry controls in Light OFF conditions.

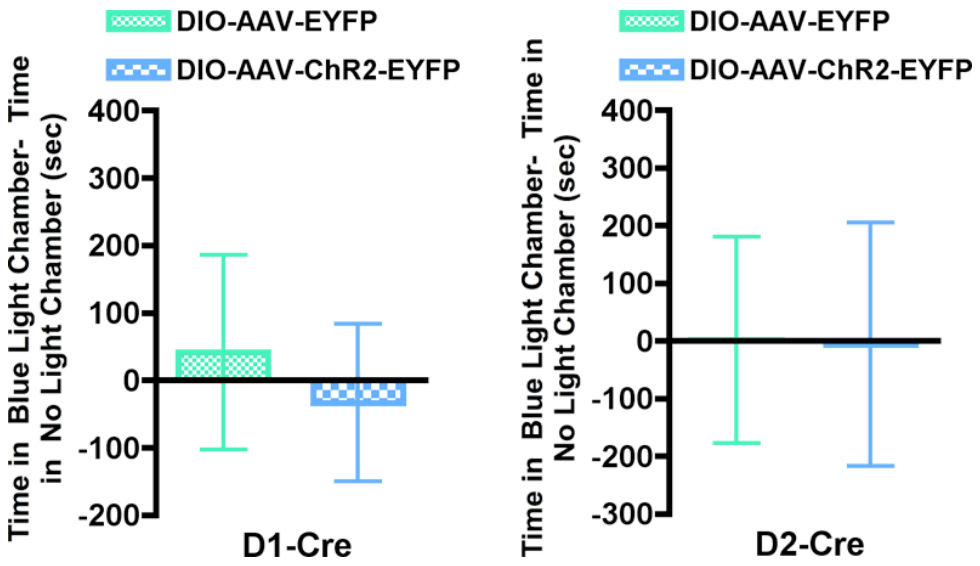

Fig S8. Absence of blue light CPP in the absence of cocaine in D1-Cre and D2-Cre DIO-AAV-ChR2-EYFP and DIO-AAV-EFYP mice. No preference or aversion for the blue light chamber was observed in the absence of cocaine.

	D1-Cre-flTrkB		D2-Cre-flTrkB		Striatal/ NAc MSN
Gene	Fold Change	p value	Fold Change	p value	Expression
Kcna2	0.60 ± 0.02	0.004	0.73 ± 0.03	0.008	ABA, *S3, S4, S5*
Kcnb1	0.56 ± 0.03	0.005	0.74 ± 0.03	0.140	ABA, *S3, S6*
Kcnd1	0.61 ± 0.09	0.02	0.85 ± 0.11	0.359	ABA, *S7*
Kcnj4	0.35 ± 0.02	0.01	2.51 ± 1.8	0.45	ABA, *S8*
Kcnj8	0.39 ± 0.02	0.01	0.63 ± 0.14	0.144	ABA

Table S1. Downregulation of K^+ channel subunits in D1-Cre-flTrkB NAc after cocaine exposure. Real time qRT-PCR analysis of potassium channels in the NAc of D1-Cre-flTrkB and D2-Cre-flTrkB mice, and their controls, after 7 days of repeated cocaine (20 mg/kg) treatment, with animals analyzed 24 hours after the last injection. Genes listed are 5 striatal/NAc enriched K^+ channels found to be significantly decreased in D1-Cre-flTrkB NAc after cocaine exposure (Student's t-test). Only one K^+ channel was similarly decreased by cocaine in the NAc of D2-Cre-flTrkB mice. Fold change values are $2^{-\Delta\Delta Ct}$ values for cocaine-treated D1-Cre-flTrkB and D2-Cre-flTrkB NAc relative to $2^{-\Delta\Delta Ct}$ values for respective cocaine treated controls. ABA, verification of striatal/NAc enrichment in the Allen Brain Atlas (www.brain-map.org).

Supplemental Methods

Mice

D1-GFP, D2-GFP, D1-Cre, and D2-Cre BAC transgenic mice on a C57Bl/6 background were obtained from N. Heintz, P. Greengard, C. Gerfen, and NINDS/GENSAT (www.gensat.org) (*S3, S4*) from Rockefeller University/NIH/NIMH. D1-Cre–flTrkB and D2-Cre–flTrkB mice on a mixed C57Bl/6 x FVB/N background were generated by crossing D1-Cre or D2-Cre mice to conditional flTrkB mice obtained from L. Parada at UT Southwestern (S5). Control mice for the D1-Cre-flTrkB and D2-Cre-flTrkB behavioral experiments were flTrkB littermates lacking the D1-Cre or D2-Cre transgene. Mice were maintained on a 12-hour light dark cycle with food and water *ad libitum*. All animal protocols were in accordance with the National Institutes of Health Guide for Care and use of Laboratory Animals and approved by the Mount Sinai Institutional Animal Care and Use Committee.

FACS, RNA extraction, and qRT-PCR

Total striatum (dorsal striatum and NAc) from D1-GFP and D2-GFP mice, age 8-9 weeks, were enzymatically dissociated and a pure population of D1-GFP+ or D2-GFP+ MSNS was obtained via fluorescence activated cell sorting (FACS) using a previously published protocol (*S1*). RNA was extracted using a micro RNeasy kit including a DNase I digestion according to the manufacturers protocol. RNA quality and relative concentration were confirmed on the Picochip using Agilent 2100 Bioanalyzer. RNA was subjected to two rounds of in vitro transcription amplification using a Riboamp HS Plus kit and cDNA was generated using this kit combined with Superscript III according to the manufacturers protocol. Quantitative PCR was carried out on a 7900HT with 4 µl of cDNA, Taqman Universal PCR mice - No AmpErase UNG, and one of the following TaqMan Gene Expression Assays: Drd1a (D1, Mm01353211_m1), Drd2 (D2, Mm00438541_m1), GAPDH (Mm03302249_g1), and Ntrk2 (TrkB, Mm00435422_m1) (ABI).

For whole NAc RNA extraction, the NAc was punched using a 14-gauge punch then processed with a micro RNeasy kit (see above). RNA was reversed transcribed into cDNA using iScript cDNA synthesis. Quantitative PCR was carried out on a 7900HT with 2 µl of cDNA, SYBR green master mix, and GAPDH or c-Fos primers. Samples were run in triplicate and data were analyzed using the $2^{-\Delta\Delta Ct}$ method, as previously described (*S6*) using GAPDH as a normalization control. Samples were also normalized to D1+ MSN samples for D1 receptor mRNA expression and D2+ MSN samples for D2 receptor and TrkB mRNA expression.

For K^+ channels qRT-PCRs, NAc was punched as described above from D1-Cre-flTrkB and D2-Cre-flTrkB mice, and their controls, 24 hours after receiving either 7 days of cocaine (20 mg/kg) or saline. RNA was extracted as described above and reverse transcribed using RT^2 First Strand Kit, then run on a RT^2 PCR Profiler Array according to manufacture's protocols. ΔCt was determined using SABiosciences software and then

normalized to each group's saline treated NAc tissue to obtain $2^{-\Delta\Delta Ct}$ values. Values in Table S1 are $2^{-\Delta\Delta Ct}$ values for cocaine-treated D1-Cre-flTrkB and D2-Cre-flTrkB NAc relative to $2^{-\Delta\Delta Ct}$ values for respective cocaine-treated controls.

Virus Vectors

DIO-AAV-ChR2-EYFP and DIO-AAV-EYFP virus plasmids (*S7*) and AAV viruses were generated according to previous protocols (*S8*). AAVs are expressed maximally ~2 weeks post infection. Mice with AAV injections in the NAc were tested in behavioral experiments 3 weeks after AAV infection. HSV-ChR2-mCherry (in HSVPrpUC) using the ChR2-mCherry DNA construct (*S9, S10*), HSV-TrkB.FL-GFP-mCherry (in p1005/mCherry) using the TrkB.FL-GFP construct, a gift from V. Lessman (*S2*), and HSV-mCherry (in p1005/mCherry) vectors were engineered by R. Neve at MIT (*S11*). Because HSV expression is maximal on day 3 and 4 post infection all ChR2 blue light stimulation was performed on these days.

Stereotaxic Surgery, Virus and Cannula Placement, and Optic Fiber Placement

Mice were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg), placed in a small-animal stereotaxic instrument, and their skull surface was exposed. For ChR2 viruses, thirty-three gauge syringe needles were used to unilaterally infuse 0.5 µl of virus into the NAc of the right hemisphere at a 0° angle (AP + 1.4; ML + 1.3; DV – 4.3) at a rate of 0.1 µl/min. Next a 20-guage cannula, 4 mm in length from the cannula base, was implanted into the right hemisphere (AP + 1.4; ML + 1.3; DV – 3.9). Instant adhesive was placed between the base of the cannula and the skull and Skull Fixture Adhesive was used to cement the cannula to the skull. For AAV surgeries, we performed two surgeries with the virus surgery first followed by the cannula surgery 3 weeks later when the AAV virus was expressed and the mice cranium had healed from the virus surgery. Mice were allowed to recover for 2 days following the surgery (virus and cannula for HSV, second cannula surgery for AAV), then used in conditioned place preference (CPP) experiments, locomotor experiments, or ChR2 test experiments.

For *in vivo* optical control of neuronal firing, a 200 µm core optic fiber was modified for attachment to the cannula. When the optic fiber was secured *in vivo* to the cannula the fiber was flush with the length of the cannula with ~50µm of the stripped 200 µm core exposed beyond the cannula. This experimental set-up was based on but modified slightly from previous studies (*S7*, *S9, S10*). Optic fibers were secured *in vivo* to the cannula head-mount only during experimental procedures.

For HSV-TrkB-GFP-mCherry and control virus similar procedures as above were used except both hemispheres were infused and the coordinates were a10° angle (AP + 1.6; ML + 1.5; DV – 4.4).

Blue Light Stimulation

Optical stimulation was performed similar to previous published protocols (*S7, S8*). Optic fibers were attached through an FC/PC adaptor to a 473 nm blue laser diode (#BCL-473-050-M), and light pulses were generated through a stimulator (#33220A). For all *in vivo* experimental protocols, 10 Hz blue light pulses over 3 min were delivered (*S9*). Optic fiber light intensity was measured using a light sensor (#S130A) and light

intensity ranged from 2-4 mW.

Electrophysiology

Whole cell recordings were obtained from NAc shell MSNs in acute brain slices from Rgs9-Cre, D1–Cre-flTrkB and D2–Cre-flTrkB mice that had been stereotaxically injected into the NAc with DIO-AAV-ChR2-EYFP or DIO-AAV-EYFP. Acute brain slices at the NAc level were prepared as described previously (*S12*). The aCSF contained the following (in mM): 128 NaCl, 3 KCl, 1.25 NaH_2PO_4, 10 D-glucose, 24 $NaHCO_3$, 2 $CaCl_2$, and 2 $MgSO_4$, oxygenated with 95% O_2 and 5% CO_2 (pH 7.35, 295–305 mOsm). Sucrose aCSF was derived by fully replacing NaCl with 254 mM sucrose in aCSF. Patch pipettes (3–5 MΩ) for whole-cell current clamp recordings were filled with an internal solution containing the following (in mM): 115 potassium gluconate, 20 KCl, 1.5 $MgCl_2$, 10 phosphocreatine, 10 HEPES, 2 magnesium ATP, and 0.5 GTP (pH 7.2, 285 mOsm). The experiments were carried out at 34ºC. The resting membrane potential and action potentials of MSNs were recorded in current-clamp mode of amplifier Multiclamp 700B and data acquisition was realized with pClamp 10. Series resistance was monitored during experiments. Responses were obtained from 1-6 neurons from each animal, with means per animal then used to generate group means and perform statistical analyses. Blue light trains (1.0 or 1.4 Hz) were generated by a stimulator and delivered to MSNs expressing ChR2 (Rgs9-Cre mice) through a 200 μm optic fiber attached to a 473 nm blue laser.

Blue Light Stimulation for c-Fos Induction

Mice (D1-Cre and D2-Cre with NAc injections of DIO-AAV-ChR2-EYFP or DIO-AAV-EYFP; C57Bl/6 wildtype mice with NAc injections of HSV-ChR2-mCherry or HSV-mCherry) were acclimated to 10-min 10 Hz 473 nm blue light pulses on day 1. On day 2, mice were again exposed to 10-min 10 Hz blue light pulses. For immunohistochemistry studies mice were anesthetized, 90 min after blue light stimulation, with chloral hydrate then perfused with 1x phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brains were post-fixed overnight in 4% PFA then cryo-protected in 30% sucrose in PBS. For further information see immunohistochemistry section below. For RNA studies, mice were sacrificed 30 min post blue light stimulation. Virus infected NAc was visualized on a fluorescent dissecting scope and 14 gauge tissue punches were taken and later extracted for RNA.

Immunohistochemistry

Mice were perfused and brains were cryo-protected according to methods above. Brains were sliced on a microtome at 35 μm (for D1-Cre–flTrkB, D2-Cre–flTrkB, and respective controls) or 40 μm (for ChR2 virus expressing mice and controls). Free floating brain sections were blocked in 3% normal donkey serum (NDS) and 0.3% Triton-X for 1 hour before adding primary antibody. For c-Fos/Cre double labeling in D1-Cre–flTrkB and D2-Cre–flTrkB, brain sections were incubated in 1:2000 of rabbit anti-c-Fos (Santa Cruz, sc-52) and 1:1000 mouse anti-Cre recombinase (Millipore, MAB3120) in block overnight at room temperature. The next day sections were rinsed in PBS then incubated in 1:500 of donkey anti-rabbit Cy2 and 1:500 of donkey anti-mouse Cy3 in PBS for 1 hour then subsequently rinsed in PBS.

For GFP (to detect EYFP expression)/Cre double labeling, sections were incubated in 1:8000 of chicken anti-GFP (Aves, #GFP 1020) and mouse anti-Cre recombinase (see above). The next day sections were processed similar to above except 1:500 donkey anti-chicken Cy2 was used. For GFP/Cre double labeling, a similar protocol was used above with the same anti-GFP and anti-Cre recombinase antibodies and their respective secondary antibodies. For c-Fos/mCherry double labeling, the same anti-c-Fos antibody was used but sections were processed first for Cre. This included a 1 hour block in 3% normal goat serum (NGS) and 0.3% Triton-X, then an overnight incubation in 1:1000 rabbit anti-c-Fos followed by detection with 1:500 of a biotinylated goat anti-rabbit antibody (Vector Labs), and then 1:1000 of Streptavidin Alexa 488 (Invitrogen). Sections were rinsed in PBS then blocked again in 3% NDS and 0.3% Triton-X followed by overnight incubation in 1:1000 of rabbit anti-dsRed (this detects mCherry), then detection with 1:500 of donkey anti-rabbit Cy3. For GFP/mCherry double labeling, sections were processed simultaneously with both antibodies according to GFP and mCherry immuno-labeling procedures above. For single detection of mCherry, sections were processed with rabbit anti-mCherry according to methods above. For c-Fos/GFP double labeling a similar protocol similar to above was used and with the same anti-c-Fos and ant-GFP antibodies and their respective secondary antibodies.

For Enkephalin/Cre double labeling, sections were blocked in 3% NGS, 3% milk, and 0.6% Tween-20. Sections were incubated in 1:250 mouse anti-Enkephalin (Millipore, #MAB350) and 1:1000 anti-rabbit Cre recombinase overnight in the same block containing only 0.3% Tween-20. The following day sections were incubated in 1:200 biotinylated goat anti-mouse then processed in 1:500 Streptavidin Cy3 and 1:500 donkey anti-rabbit Cy2.

All sections were mounted onto superfrost plus slides, allowed to dry then dehydrated in alcohols and cleared in Citrisolv, then coverslipped in DePex. Sections were subsequently imaged on a LSM 710 confocal (Zeiss).

Western Blots

Mice were exposed to blue light for two consecutive days for 3x 3min 10 Hz pulses within a 15 min period. 30 min after the stimulation, on day 3, NAc infected virus tissue was visualized on a fluorescent dissecting scope then dissected with a 14-gauge punch. NAc tissues were homogenized in 30 μL of homogenization buffer containing 320 mM sucrose, 5 nM Hepes buffer, 1% SDS, phosphatase inhibitor cocktails I and II and protease inhibitors using an ultrasonic processor. Protein concentrations were determined using a DC protein assay and 30 μg protein was loaded onto 4-15% gradient Tris-HCl polyacrylamide gels for electrophoresis fractionation. The samples were then transferred to a nitrocelluose membrane and blocked for one hour in Odyssey blocking buffer. Blocked membranes were incubated overnight at 4°C with primary antibodies (1:500 phospho-ERK44/42, 1:1000 ERK44/42, Cell Signalling) in Odyssey blocking buffer. After thorough washing with TBST, the membrane was incubated with respective IRDye secondary antibodies (1:5000 to 1:10000) dissolved in Odyssey blocking buffer for 1 hour at room temperature. The blots were imaged with the Odyssey Infrared Imaging system and quantified by ImageJ. The amount of protein blotted onto each lane was normalized to levels of tubulin (1: 60000, Millipore).

Cell Counting

For c-Fos/Cre cell counting analysis in D1-Cre–flTrkB and D2-Cre–flTrkB and their controls, mice were given 6 days of saline ip injections to acclimate them to the injections. On day 7, mice were given an acute cocaine (20 mg/kg) injection or saline injection then perfused (see above) 90 min after the injection. Mouse brains sectioned at 35μm and were processed for Cre/c-Fos double immuno-labeling (see above). Images sampling Bregma 1.5-1.1 of the NAc (core and shell) and dorsal striatum were taken using a 63x water objective (135 μm x 135 μm) on an LSM 710 confocal. Cells were counted using ImageJ software. Approximately >200 and > 400 Cre positive neurons were counted in the, D1-Cre-flTrkB and D1-Cre controls and D2-Cre-flTrkB and D2-Cre controls respectively, NAc and then the number of c-Fos positive neurons were counted within those groups. For bregma diagrams and raw cell numbers see Fig S6.

For enkephalin/Cre cell counting 200 μm by 200 μm images of NAc (core and shell) and dorsal striatum, from D1-Cre and D2-Cre mice were, scanned and 35 Cre positive neurons were counted per animal per area and then the number of enkephalin positive neurons were counter within this group.

For c-Fos quantification in the ChR2 conditions, 200μm by 200μm images of NAc virus infected region were scanned and 50 ChR2 positive neurons were counted for c-Fos reactivity per animal. Similar methods were employed for quantification of HSV-ChR2-mCherry positive neurons in D1-GFP and D2-GFP mice.

Conditioned Place Preference

We used an unbiased CPP paradigm similar to a previously described study (*S13*). Mice were evaluated for cocaine or cocaine/blue light CPP in a 3-chamber CPP box using Med Associates software. The box consisted of a smaller middle chamber and two conditioning larger chambers with different contextual clues including a gray verses stripe chamber, different floor mesh, and different lighting. Mice were allowed to explore the 3 chambers for 20 min and those that showed significant preference for one of the two conditioning chambers, were excluded from the study (<10% of all animals). Groups were then balanced and adjusted for any chamber bias that may still exist.

For standard cocaine CPP, D1-Cre–flTrkB and D2-Cre–flTrkB mice and their respective littermate controls were conditioned for two days to saline injections (ip) in one chamber for 30 min during a morning session and 7.5 mg/kg cocaine injections (ip) to the opposite chamber for 30 min during an afternoon session. The day after the second conditioning, mice were tested for place preference during a 20-min session where they were allowed to freely explore all 3 chambers.

For cocaine/blue light CPP, optic fibers were secured to the cannula prior to saline or cocaine ip injections. Mice were conditioned to saline and no light for a 30-min session and cocaine (7.5 mg/kg for D2-Cre DIO-AAV-ChR2 EYFP and their controls or 5 mg/kg for D1-Cre DIO-ChR2-EYFP and C57Bl/6 wildtype mice given HSV-ChR2-mCherry, and their respective controls) and blue light for a 30-min session over two days. 10 Hz 473 nm blue light pulses were delivered into the NAc for four 3-min periods during the 30-min session. We chose the 10 Hz frequency because a previous study used this frequency to assess ChR2-blue light place preference, in the absence of drugs, and

although this frequency was not significant it exhibited a trend for increased place preference compared to a 3.8 Hz frequency (*S9*). Mice were tested for place preference as described above. For blue light only CPP mice were conditioned as above but in the absence of saline and cocaine. For all groups, baseline locomotion in response to saline was assessed to ensure that locomotion was not affected by viral treatment or cannula/optic fiber placement. All mice displayed normal crossovers between the chambers. For all experiments, CCP scores are expressed as time spent in cocaine chamber minus time spent in saline chamber. To compare HSV-TrkB.FL-EGFP-mCherry rescue CPP to the TrkB knockout CPP we performed data analysis similar to previously published results (*S13*), by normalizing D1-Cre-flTrkB and D2-Cre-flTrkB scores to their control group scores resulting in % Control.

Locomotor Activity and Open Field

Baseline locomotor and open field activity in a novel chamber in D1-Cre-flTrkB and D2-Cre-flTrkB mice and controls were performed in 75 cm^3 chambers using Ethovision XT. Locomotor activity for D1-Cre–flTrkB and D2-Cre–flTrkB was performed according to previous studies (*S14*) but with the following alterations. Activity was assessed in rat-sized cages set up in locomotor apparatuses and mice were evaluated for horizontal ambulations on the x and y plane. On day 0, mice were acclimated to the novel environment for 30 min and then to saline (ip injections) for 30 min. Mice were given ip Injections of cocaine (10 mg/kg) on day 1 through 7 immediately placed into the locomotor apparatus and assessed for locomotor activity over a 30-min period on each day.

Locomotion for ChR2 studies in D1-Cre and D2-Cre mice was assessed in home cages using Ethovision XT. Cocaine naive mice were acclimated in home cages in the novel room for 15 min with the optic fiber secured to their head-mount. Mice were then tested for locomotor activity (distance moved in cm) with three 3-min 10 Hz blue light pulses in a 15-min time period. The next day mice were given repeated doses of 15 mg/kg cocaine (i.p. injections) for 6 days. 1 day after the last dose of cocaine mice were evaluated for locomotor activity during blue light stimulation (see above).

Statistical Analysis

Two-way repeated measures ANOVAs and one-way repeated measures ANOVAs were used to calculate statistics for locomotor activity of D1Cre-flTrkB and D2Cre-flTrkB mice and their controls. Two-Way ANOVAs were used to calculate statistics in D1-Cre and D2-Cre ChR2 locomotor assays. Student's t-tests (2 way, 2 tailed) were used as a post-hoc analysis for both locomotor experiments. All other experiments, CPP and cell counting experiments used Students t-tests (2 way, 2 tailed).

Supplemental References:

S1. M. K. Lobo, S. L. Karsten, M. Gray, D. H. Geschwind, X. W. Yang, *Nat Neurosci* **9**, 443 (2006).
S2. M. Hartmann *et al., J Cell Sci* **117**, 5803 (2004).
S3. Z. Bekele-Arcuri et al., *Neuropharm* **35**, 851 (1996).
S4. Y.H. Chung *et al*., *Brain* Res **875**,164 (2000).
S5. W. Shen *et al., J Neurophysiol* **91**, 1337 (2004).
S6. M.A. Ariano *et al*., J Neurophysiol **93**, 2565 (2005).
S7. T. Tkatch *et al*., *J Neurosci* **20**, 579 (2000).
S8. P.G. Mermelstein et al., *J Neurosci* **18**, 6650 (1998).
S3. S. Gong *et al., J Neurosci* **27**, 9817 (2007).
S4. S. Gong *et al.*, *Nature* **425**, 917 (2003).
S5. B.W. Luikart, S. Nef, T. Shipman, L. F. Parada, *Neuroscience* **117**, 847 (2003).
S6. W. Renthal *et al.*, *Neuron* **56**, 517 (2007).
S7. J. A. Cardin *et al.*, *Nat Protoc* **5**, 247 (2010).
S8. J. D. Hommel, R. M. Sears, D. Georgescu, D. L. Simmons, R. J. DiLeone, *Nat Med* **9**, 1539 (2003).
S9. R. D. Airan, K. R. Thompson, L. E. Fenno, H. Bernstein, K. Deisseroth, *Nature* **458**, 1025 (2009).
S10. V. Gradinaru *et al., J Neurosci* **27**, *52* (2007).
S11. R. L. Neve, K. A. Neve, E. J. Nestler, W. A. Carlezon, Jr., *Biotechniques* **39**, 381 (2005).
S12. D.L. Wallace, *et al*., *Nat Neurosci* **12**, 2 (2009).
S13. I. Maze *et al., Science* **327**, 213 (2010).
S14. M. H. Han *et al.*, *Biol Psychiatry* **67**, 761 (2010).