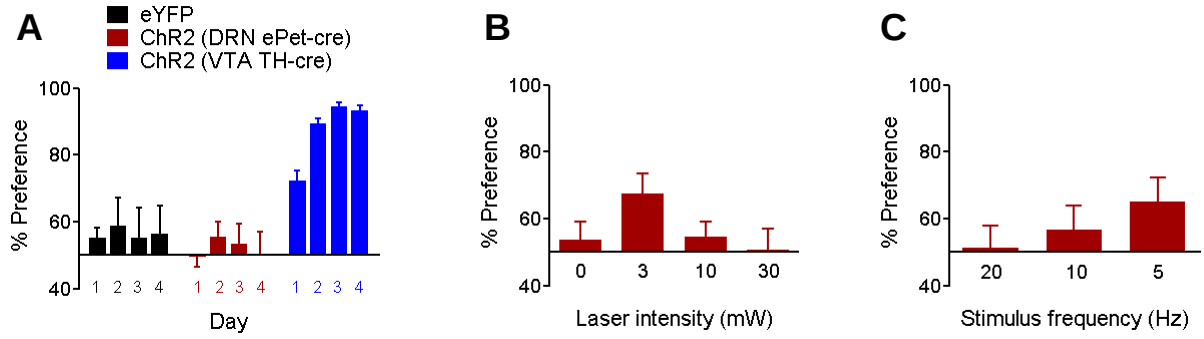

Figure S1, related to Figure 2. Parametric variations of laser stimulus do not result in statistically significant preference or avoidance of laser-paired chamber in mice with optogenetic stimulation of DRN serotonin neurons. A, Mice were tested on four consecutive days for real-time place preference of optogenetic stimulation on one half of a chamber. Stimulation was delivered at 15 mW strength, in 5 ms pulses at 20 Hz. Serotonin-stimulated ePet-cre mice received DRN injections of cre-ON ChR2-eYFP (*n*=18). Also included were positive control mice with stimulation of VTA dopamine neurons via cre-ON ChR2-eYFP in TH-cre mice (*n*=10) and negative control mice receiving cre-ON eYFP alone, injected into DRN or VTA (*n*=10). B, One cohort of DRN serotonin-stimulated mice (*n*=10) was tested in "context B," a chamber with different markings from the one used in the repeated-days experiment in panel A. Testing was conducted one week after nose-poke self-administration experiment (Figure 2F). Laser intensity was increased in four consecutive daily tests, with stimulation delivered in 5 ms pulses at 20 Hz. C, A second cohort of DRN serotonin-stimulated mice (*n*=8) were tested in context B, as described above. Laser stimulus was applied at 15 mW in 5 ms pulses, with frequency varying in daily tests.

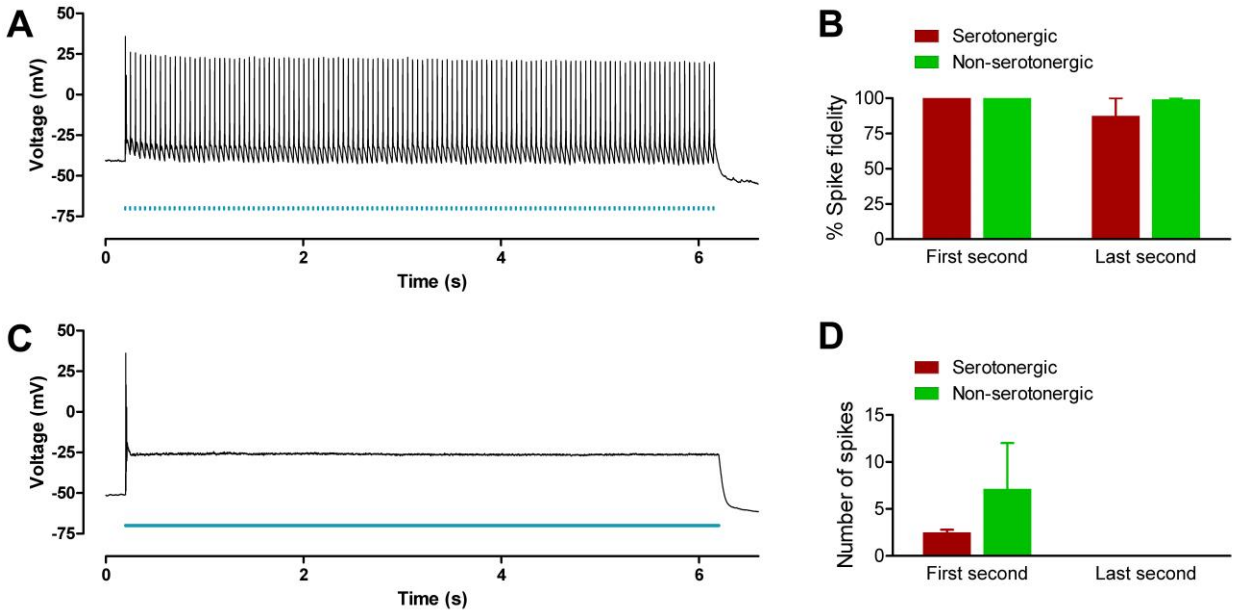

Figure S2, related to Figure 2. Serotonergic DRN neurons do not enter depolarization block with 20 Hz optogenetic stimulation. A, DRN neurons from mice injected with nonspecific ChR2 virus were recorded in current clamp, as in Figure 2J. When stimulated with 20 Hz 5-msec pulses of blue light, neurons followed with action potentials throughout the 6-second stimulation period. B, Spike fidelity in the first and last second of the stimulation period did not differ between putative serotonergic (*n*=4 cells; 4 mice) and non-serotonergic (*n*=6 cells; 5 mice) neurons, identified by presence or absence of hyperpolarization in response to bath application of 100 µM serotonin at the end of the experiment, a response mediated by 5-HT_{1A} receptors that are a preferential marker of serotonergic identity within the DRN (Day et al., 2004; Kirby et al., 2003). C, In contrast to pulsed light, DRN neurons respond to constant illumination by initial spiking, followed by depolarization without action potentials. D, In all cells examined, spikes were present in the first but not in the last second of a 6-second period of illumination.

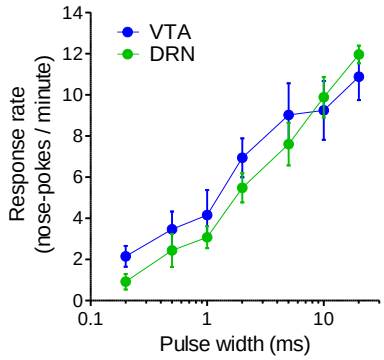

Figure S3, related to Figure 3. Optical self-stimulation for VTA dopamine cell bodies and non-serotonergic DRN cell bodies demonstrate similar input-output curves. Well-trained mice were given access to self-stimulation for VTA dopamine cell bodies (n=7) or DRN non-serotonin cell bodies (n=5). Mice were placed in operant chambers and allowed to nose-poke for 3-second trains of 20 Hz 5ms pulses of light for a baseline period of 10 minutes. Afterwards, mice were given 5 minutes of access to nose-poking for trains with descending pulse widths, from 20 to 0.2 ms.

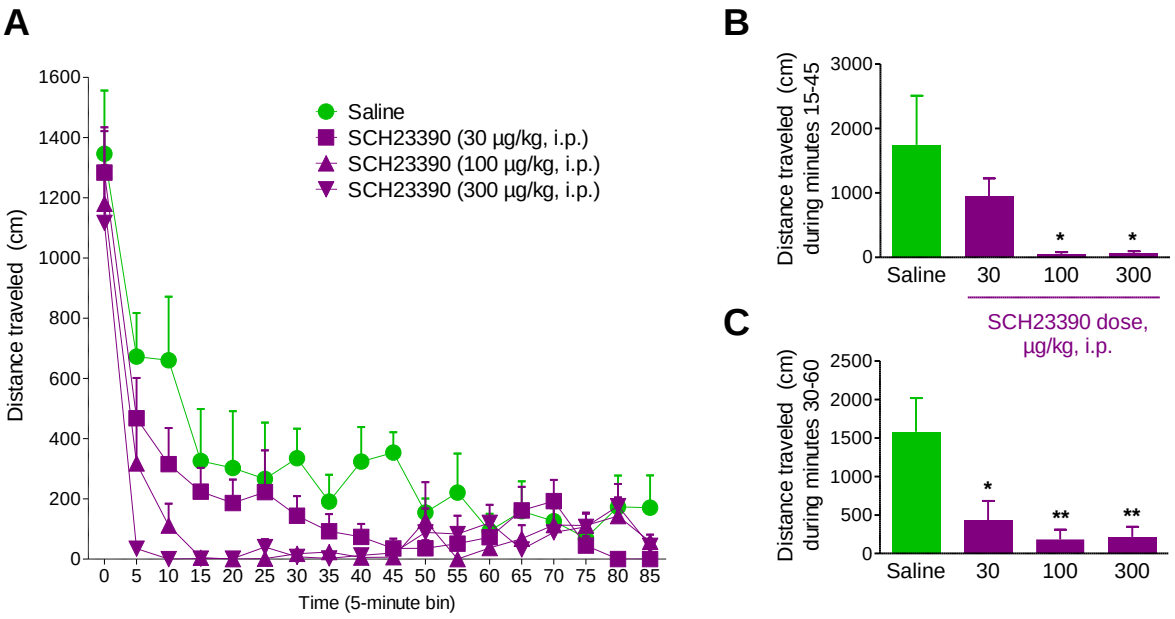

Figure S4, related to Figure 3. SCH23390 dose-dependently reduces novelty-induced locomotion. Naïve wild-type mice were injected with saline or the dopamine D1 antagonist SCH23390 at 30, 100, or 300 µg/kg, i.p. (*n*=4/group) and immediately placed in a novel environment, consisting of a plastic 33 x 40 cm tub containing fresh bedding. Novelty-induced locomotion was monitored for 90 minutes A, Distance traveled during 5-minute bins. Panels B and C depict drug effects during time periods used in nose-poke experiment in Figure 3. B, Distance traveled during minutes 15-45 after injection. One-way ANOVA $F_{(3,12)}$=3.966, p<0.05; * p<0.05 Dunnett's post-hoc vs saline. C, Distance traveled during minutes 30-60 after injection. One-way ANOVA $F_{(3,12)}$=6.080, p<0.01; * p<0.05, ** p<0.01 Dunnett's post-hoc vs saline.

Promoter	Gene	Floxed?	AAV serotype	Shorthand
EF1α	eYFP	Double inverted open reading frame (DIO), aka “FLEX”	1	cre-ON eYFP
EF1α	ChR2-eYFP	DIO	1	cre-ON ChR2-eYFP
CMV	cre-GFP	-	1	cre
EF1α	ChR2-eYFP	loxP-flanked	1	cre-OFF ChR2-eYFP
Synapsin	ChR2-eYFP	-	5	ChR2

Table S1, related to Figures 2,3,4,5,7. List of viral vectors used in experiments.

Adeno-associated virus (AAV) vectors are listed in the order they are introduced in the manuscript. All ChR2 constructs contain the H134R mutation to increase amplitude of currents (Nagel et al., 2005). Cre-ON vectors contain a double-inverted open reading frame (Atasoy et al., 2008). Cre-OFF vectors contain a loxP-flanked open reading frame, use a similar strategy to other recently characterized vectors (Saunders et al., 2012). AAV-1 serotypes were packaged in-house by the National Institute on Drug Abuse Optogenetics and Transgenic Technology Core. AAV-5 vector was packaged at the University of North Carolina Vector Core.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Electrophysiology: Animals were anesthetized and perfused with ice-cold artificial cerebrospinal fluid (ACSF). 250 µm horizontal VTA-containing or coronal DRN-containing slices were prepared in ice-cold ACSF. Following cutting, slices were recovered for 10 minutes at 34ºC and then transferred to holding ACSF at room temperature. ACSF used for perfusion, cutting, and recovery contained NMDG as a sodium substitute to preserve neuronal health (Ting et al., 2014) and contained, in mM, 92 NMDG, 20 HEPES (pH 7.35), 25 glucose, 30 sodium bicarbonate, 1.2 sodium phosphate, 2.5 potassium chloride, 5 sodium ascorbate, 3 sodium pyruvate, 2 thiourea, 10 magnesium sulfate, 0.5 calcium chloride. ACSF used for holding slices prior to recording was identical, but contained 92 mM sodium chloride instead of NMDG and contained 1 mM magnesium chloride and 2 mM calcium chloride. ACSF used to perfuse slices during recording was maintained at 31ºC and contained, in mM, 125 sodium chloride, 2.5 potassium chloride, 1.25 sodium phosphate, 1 magnesium chloride, 2.4 calcium chloride, 26 sodium bicarbonate, and 11 glucose. All ACSF preparations were saturated with 95% O2 and 5% CO2. Cells were visualized on an upright microscope with infrared differential interference contrast and fluorescence microscopy. Whole-cell patch-clamp recordings were made using a MultiClamp 700B amplifier using 2 kHz low-pass Bessel filter and 10 kHz digitization with pClamp 10.3 software (Molecular Devices, Sunnydale, CA). Cells were patched using glass pipets with resistance 2.0-3.0 MΩ, filled with internal solution containing, in mM, 135 potassium gluconate, 10 HEPES (pH 7.35), 4 potassium chloride, 4 Mg-ATP, 0.3 Na-GTP. Dopaminergic neurons in the VTA were identified by morphology, tonic spike rate, and presence of a

hyperpolarization-induced Ih current, which can be a reasonable predictor of dopaminergic identity in mice (see main text). During recordings, series resistance was monitored during recording with 5 mV hyperpolarizing pulses and maintained below 30 MΩ. Cells were optically stimulated once every 30 seconds with 5 mW, 473 nM laser light via a 62.5 µM-core fiber, submerged in the bath and aimed at the region of interest. In evoked EPSC experiments, cells were held at -70 mV and external bath solution contained 100 µM picrotoxin. To confirm glutamatergic composition of currents, cells were held stable for 10 minutes baseline and then perfused with 10 µM CNQX and 50 µM APV for 10 minutes. The last 5 minutes of each period were used for data analysis. In current-clamp DRN-VTA experiments, cells were all brought to an initial resting membrane potential of -55 mV (which corresponded to average measured resting potential of several VTA dopamine neurons) prior to optical stimulation of DRN-VTA inputs. Spike fidelity current-clamp experiments were conducted without injecting current.

Surgical procedures: Surgical procedures were carried out as previously described (Britt et al., 2012a). Anesthetized mice were injected with 0.5 µl of virus (vector details available in Table S1) targeted to the midline DRN or bilaterally to the VTA through a 29-gauge needle, followed by chronic implantation of a 200 µm core fiber optic cable 0.5mm above the injection site. Fibers were threaded through 1.25mm-wide zirconia ferrules (Britt et al., 2012b). Behavior experiments for cell body stimulation began 4 weeks after surgery. After behavioral testing was complete, injection sites were confirmed histologically. Mice with no eYFP expression or center of virus/fiber target outside of the boundaries of VTA or DRN were excluded from analysis. For retrograde

experiments, mice were infused with tracer by iontophoresis to avoid damaging fibers of passage. Glass pipettes were pulled to an inner diameter of 30 µm and filled with a 1% Fluoro-Gold solution in 0.1M sodium cacodylate buffer (pH 7.5). After placing electrode tip in the VTA or SNr, a current generator (Kation Scientific; Minneapolis, MN) delivered electrical current of 1.0 µA in 7-second ON / 7-second OFF pulses over 15 minutes.

Conditioned place preference: Mice were tested for baseline preference in a 3-chamber CPP device (Med Associates; St Albans, VT) in a 15-minute session. Groups and chamber assignments were made to equalize baseline preference for drug vs saline side. Three days after baseline, mice were conditioned once per day for four days, with drug or saline given on alternating days. The range of fenfluramine doses was selected to cover a range of behaviorally relevant doses (Young et al., 2006). Immediately following injection, mice were restricted to one chamber for 20 minutes. On the last day, mice were placed in chambers with free access for 15 minutes. Percent preference was calculated as (time in drug side) / (time in drug side + time in saline side).

Intravenous self-administration: Standard self-administration methods were used to assess the rewarding effects of amphetamine and fenfluramine in mice. Intravenous catheterization surgery and the self-administration procedures are the same as described previously (Xi et al., 2011). Briefly, wild-type C57Bl6/J mice were initially trained for oral sucrose (5% solution) self-administration for 5 days to facilitate lever-pressing. Animals were then prepared for experimentation by surgical catheterization of the right external jugular vein. After 5-7 days of recovery from surgery, each mouse was placed into a test chamber (Med Associates) and allowed to lever-press for i.v. D-amphetamine (0.05 mg/kg/infusion) or D,L-fenfluramine (0.03 mg/kg/infusion). Doses

were chosen based on preliminary experiments indicating that they produced maximal lever-pressing. Each lever press led to a delivery of 0.015 ml of the drug solution over 4.2 seconds under an FR1 reinforcement schedule. Each session lasted 3 hr or until the animal received a maximally allowed 100 sucrose deliveries / 50 drug infusions. Daily drug self-administration continued until stable day-to-day operant behavior was established with a steady behavioral response pattern for at least 3 consecutive days. Mice then underwent 6 days of withdrawal from drug self-administration, followed by 7 days of drug extinction in which lever presses resulted in saline infusions.

Histology: Standard immunohistochemical procedures were used. Primary antibodies used were mouse anti-Tryptophan hydroxylase (catalog #T0678, 1:500, Sigma Aldrich; St Louis, MO), mouse anti-tyrosine hydroxylase (catalog #T1299, 1:5,000, Sigma Aldrich), rabbit anti-FluoroGold (catalog #AB153, 1:50,000, Millipore; Billerica, MA), and rabbit anti-serotonin (catalog #20080, 1:10,000, Immunostar; Hudson, WI). Images were acquired on a confocal microscope (Olympus, Center Valley, PA). Double-labeling was counted in z-stacks of 20X tissue scans, using levels of tissue in which antibody penetration was clearly visible. To quantitatively assess fluorescence intensity (Figure 5), all sections were acquired using identical parameters. In the anterograde tracing experiment, brains were cut in half coronally, just anterior to the VTA, for the purpose of mounting on a vibratome stage. Because of this, a certain amount of tissue was lost during slicing and unavailable for analysis, spanning approximately 0.5 – 2.5mm posterior to bregma. In the Fluoro-Gold experiment (Figure 6), animals were perfused one week after surgery. 50 µm slices of DRN were collected, and every 4^{th} slice was stained with antibodies against Fluoro-Gold and tryptophan hydroxylase. We performed

sequential immunohistochemistry for anti-Fluoro-Gold, followed by anti-tryptophan hydroxylase to prevent cross-reaction of antibodies, which we observed in preliminary experiments. Because we only analyzed every 4th slice of brain tissue, cell counts were multiplied by 4 to estimate total number of DRN cells (Figure 4C).

Statistical analysis: Experiments comparing a single measure of data between two groups were analyzed with a two-tailed Student's t-test. When analysis of variance (ANOVA) revealed significant group differences for multiple groups, post-hoc comparisons were performed using two-tailed Student's t-tests with Bonferroni correction, unless otherwise stated. A statistical cutoff of $p<0.05$ was used in all experiments. All graphical depictions of group data are presented as mean ± SEM.

AAV vector construction: The cDNA encoding hChR2-eYFP was PCR amplified from pAAV-EF1 α -DIO-hChR2(H134R)-EYFP-WPRE-HGHpA (Addgene 20298, Deisseroth Lab) using loxP-linkered oligos ("KpnI loxP ChR For:TCA GGT GTC GTG AGG TAC CAT AAC TTC GTA TAG CAT ACA TTA TAC GAA GTT ATG CTA GCC ACC ATG GAC TAT GGC" and "EcoRI loxP mCherry Rev: GCT TGA TAT CGA ATT CAT AAC TTC GTA TAA TGT ATG CTA TAC GAA GTT ATG GCG CGC CTT ACT TGT ACA GCT CGT C"). The amplified floxed insert was recombined using In-Fusion cloning mix (Clontech, Mountain View, CA) into Addgene 20298 that had been digested with KpnI and EcoRI restriction enzymes. A sequence-confirmed pAAV EF1α floxed ChR2(H134R)-EYFP (Addgene 50834) plasmid was used for production of AAV serotype 1 vector by modified triple transfection method (Howard et al., 2008) followed by affinity chromatography purification. Rat primary cortical neurons were prepared as described previously and transduced on day 6 in vitro with AAV1 EF1α -floxed

ChR2(H134R)-EYFP alone or co-transduced with AAV EF1 α -Cre. Nine days later, cells were washed in PBS, fixed with 4% paraformaldehyde and counterstained with DAPI to identify nuclei. Cultures were imaged for YFP and DAPI using TE2000 inverted microscope equipped with epifluorescence and YFP and DAPI filter sets, respectively (Nikon, Tokyo, Japan).

SUPPLEMENTAL REFERENCES

Atasoy, D., Aponte, Y., Su, H.H., and Sternson, S.M. (2008). A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. J Neurosci 28, 7025-7030.

Britt, J.P., Benaliouad, F., McDevitt, R.A., Stuber, G.D., Wise, R.A., and Bonci, A. (2012a). Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. Neuron 76, 790-803.

Britt, J.P., McDevitt, R.A., and Bonci, A. (2012b). Use of channelrhodopsin for activation of CNS neurons. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al] Chapter 2, Unit2 16.

Day, H.E., Greenwood, B.N., Hammack, S.E., Watkins, L.R., Fleshner, M., Maier, S.F., and Campeau, S. (2004). Differential expression of 5HT-1A, alpha 1b adrenergic, CRF-R1, and CRF-R2 receptor mRNA in serotonergic, gamma-aminobutyric acidergic, and catecholaminergic cells of the rat dorsal raphe nucleus. J Comp Neurol 474, 364-378.

Howard, D.B., Powers, K., Wang, Y., and Harvey, B.K. (2008). Tropism and toxicity of adeno-associated viral vector serotypes 1, 2, 5, 6, 7, 8, and 9 in rat neurons and glia in vitro. Virology 372, 24-34.

Kirby, L.G., Pernar, L., Valentino, R.J., and Beck, S.G. (2003). Distinguishing characteristics of serotonin and non-serotonin-containing cells in the dorsal raphe nucleus: electrophysiological and immunohistochemical studies. Neuroscience 116, 669-683.

Nagel, G., Brauner, M., Liewald, J.F., Adeishvili, N., Bamberg, E., and Gottschalk, A. (2005). Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. Current biology : CB 15, 2279-2284.

Saunders, A., Johnson, C.A., and Sabatini, B.L. (2012). Novel recombinant adeno-associated viruses for Cre activated and inactivated transgene expression in neurons. Frontiers in neural circuits 6, 47.

Ting, J.T., Daigle, T.L., Chen, Q., and Feng, G. (2014). Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. Methods in molecular biology 1183, 221-242.

Xi, Z.X., Peng, X.Q., Li, X., Song, R., Zhang, H.Y., Liu, Q.R., Yang, H.J., Bi, G.H., Li, J., and Gardner, E.L. (2011). Brain cannabinoid CB(2) receptors modulate cocaine's actions in mice. Nat Neurosci 14, 1160-1166.

Young, R., Rothman, R.B., Rangisetty, J.B., Partilla, J.S., Dukat, M., and Glennon, R.A. (2006). TDIQ (5,6,7,8-tetrahydro-1,3-dioxolo[4,5-g]isoquinoline) inhibits the consumption of "snacks" in mice. Pharmacology, biochemistry, and behavior 84, 74-83.