SUPPLEMENTAL FIGURES

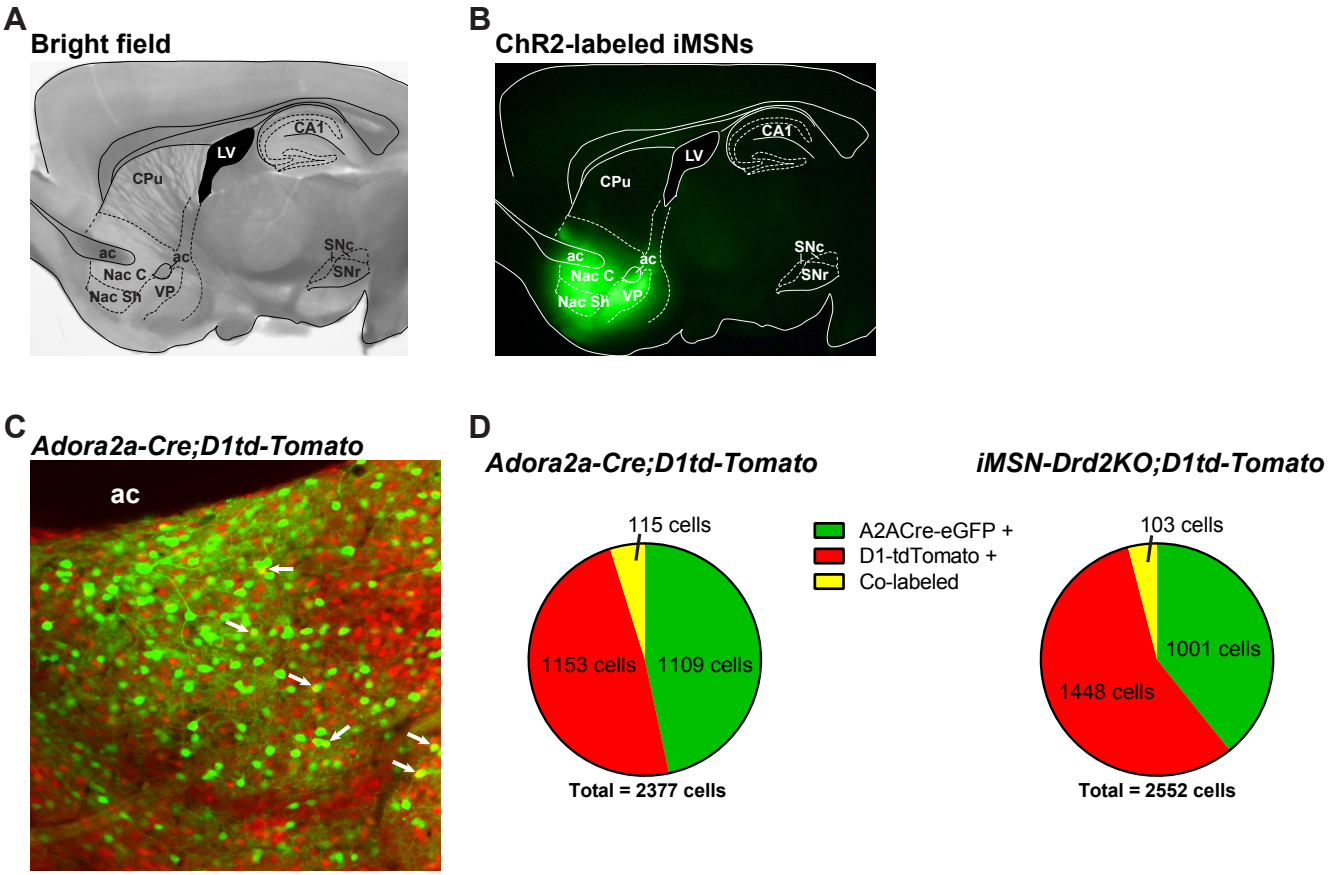

Figure S1, related to Figure 1: ChR2 expression is targeted to indirect pathway MSNs in the NAc by using Adora2a-Cre mice. A, Bright field image of a parasagittal section taken from an *Adora2a-Cre* mouse injected with an AAV expressing Cre-dependent ChR2-eYFP. **B**, Fluorescent image showing extent of ChR2 expression labeling indirect pathway MSNs in the NAc and their projections to the ventral pallidum (VP). Note the lack of ChR2 expression in the direct pathway and substantia nigra pars reticulata and pars compacta (SNr, SNc). **C**, Representative slice image containing the NAc of an Adora2a-Cre;D1td-Tomato mouse expressing eGFP in D2R-containing iMSNs. White arrows indicate MSNs co-labeled for D1-tdTomato and eGFP. D, Quantification of eGFP positive, D1td-Tomato positive, and co-labeled MSNs in the NAc of Adora2a-Cre;D1td-Tomato (left) and iMSN-Drd2KO;D1td-Tomato mice (right). ac: anterior commissure, Nac C: nucleus accumbens core, Nac Sh: nucleus accumbens shell, LV: lateral ventricle, CA1: CA1 region of the hippocampus.

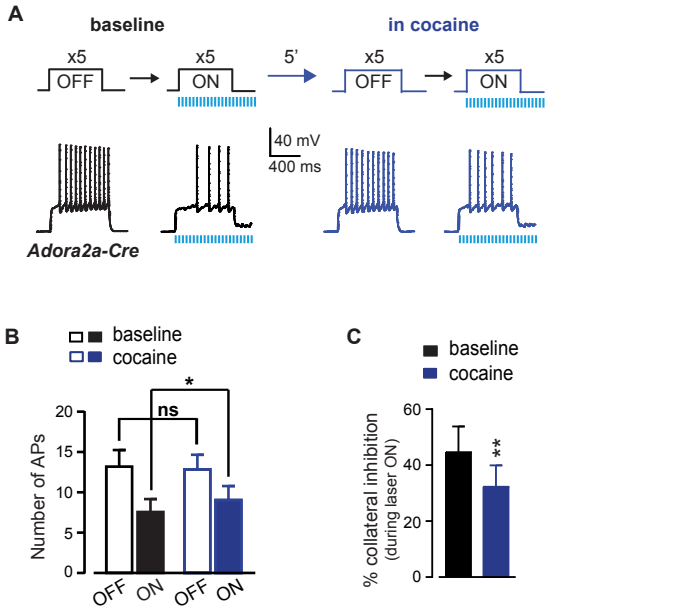

Figure S2, related to Figure 4: Cocaine disinhibits action potential firing in dMSNs. A, Design of experiment (top). Representative AP traces in *Adora2a-Cre* at baseline (black) and in the presence of cocaine (blue) during laser OFF and ON (bottom). **B**, Elicited APs at baseline (black) and in the presence of cocaine (10 μM, blue) during laser OFF (open) and ON (filled) (n = 6 cells) (2WRM ANOVA; laser x cocaine: $F_{1,5} = 11.73$, *$p < 0.05$; post-hoc: $p < 0.05$ baseline vs. cocaine at ON; p = not significant baseline vs. cocaine at OFF). **C**, Percent collateral inhibition of APs at baseline (black) or in the presence of cocaine (blue) (paired t-test: $t_5 = 6.1$, **$p < 0.01$). All data expressed as mean ± s.e.m.

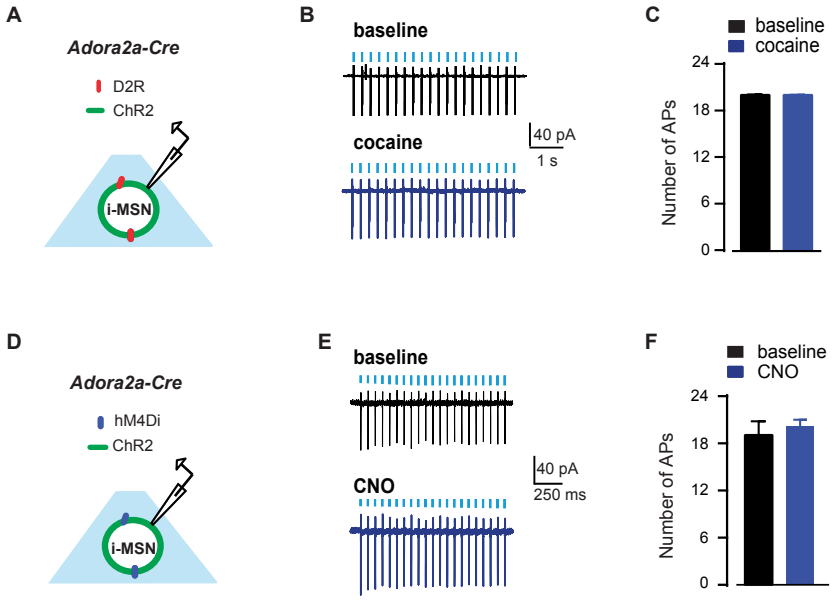

Figure S3, related to Figures 4, 7: Cocaine or CNO does not affect action potential firing in iMSNs. A, Schematic of experimental cell-attached configuration recording in iMSNs for the cocaine experiment. **B**, Representative AP traces recorded from iMSNs in *Adora2a-Cre* at baseline (black) and in the presence of cocaine (10 μM, blue). **C**, Elicited APs with 20 pulse light stimulation delivered at 4 Hz at baseline (black) and in the presence of cocaine (blue) (n = 17 cells, paired t-test, $p > 0.05$). **D**, Schematic of experimental cell-attached configuration recording in iMSNs expressing hM4Di for the CNO experiment. **E**, Representative AP traces recorded from iMSNs in *Adora2aCre* at baseline (black) and in the presence of CNO (1 μ M, blue). **F**, Elicited APs with 20 pulse light stimulation delivered at 16 Hz at baseline (black) and in the presence of CNO (blue) (n = 6 cells, paired t-test,

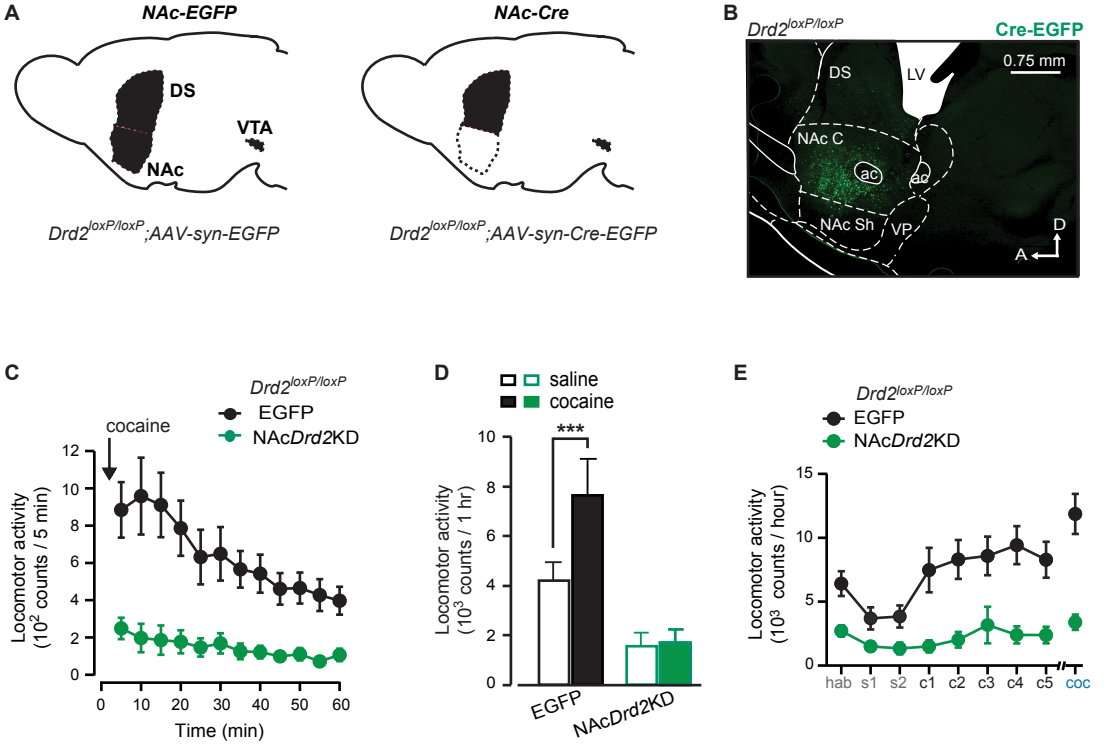

Figure S4, related to Figures, 4, 6: D2Rs in the NAc are necessary for acute cocaine locomotion. A, Schematic of D2R expression in the NAc, DS and VTA of *Drd2*$^{loxP/loxP}$ mice expressing EGFP (EGFP, left) or with a Cre-induced knockdown of D2Rs (NAcDrd2KD, right). **B**, Fluorescent staining of Cre-EGFP (green) in the NAc of a *Drd2*$^{loxP/loxP}$ mouse. NAc C: NAc core, NAc Sh: NAc shell, VP: ventral pallidum, ac: anterior commissure, LV: lateral ventricle. **C**, Timecourse of cocaine locomotion in NAcDrd2KD (n = 10, green) and EGFP littermates (n = 12, black). **D**, Locomotor activity per hour after saline (open) or cocaine (filled) (2WRM ANOVA; vector x cocaine: $F_{1,20} = 8.38$, $p < 0.01$; post-hoc: *** $p < 0.001$ cocaine vs. saline in EGFP mice). **E**, Locomotor activity to a cocaine sensitization protocol in NAcDrd2KD and EGFP controls (2WRM ANOVA; day x genotype: $F_{8,80} = 3.65$, $p < 0.01$; post-hoc: $p > 0.05$ cocaine 1 vs. challenge for NAc- Drd2KD). All data expressed as mean ± s.e.m.

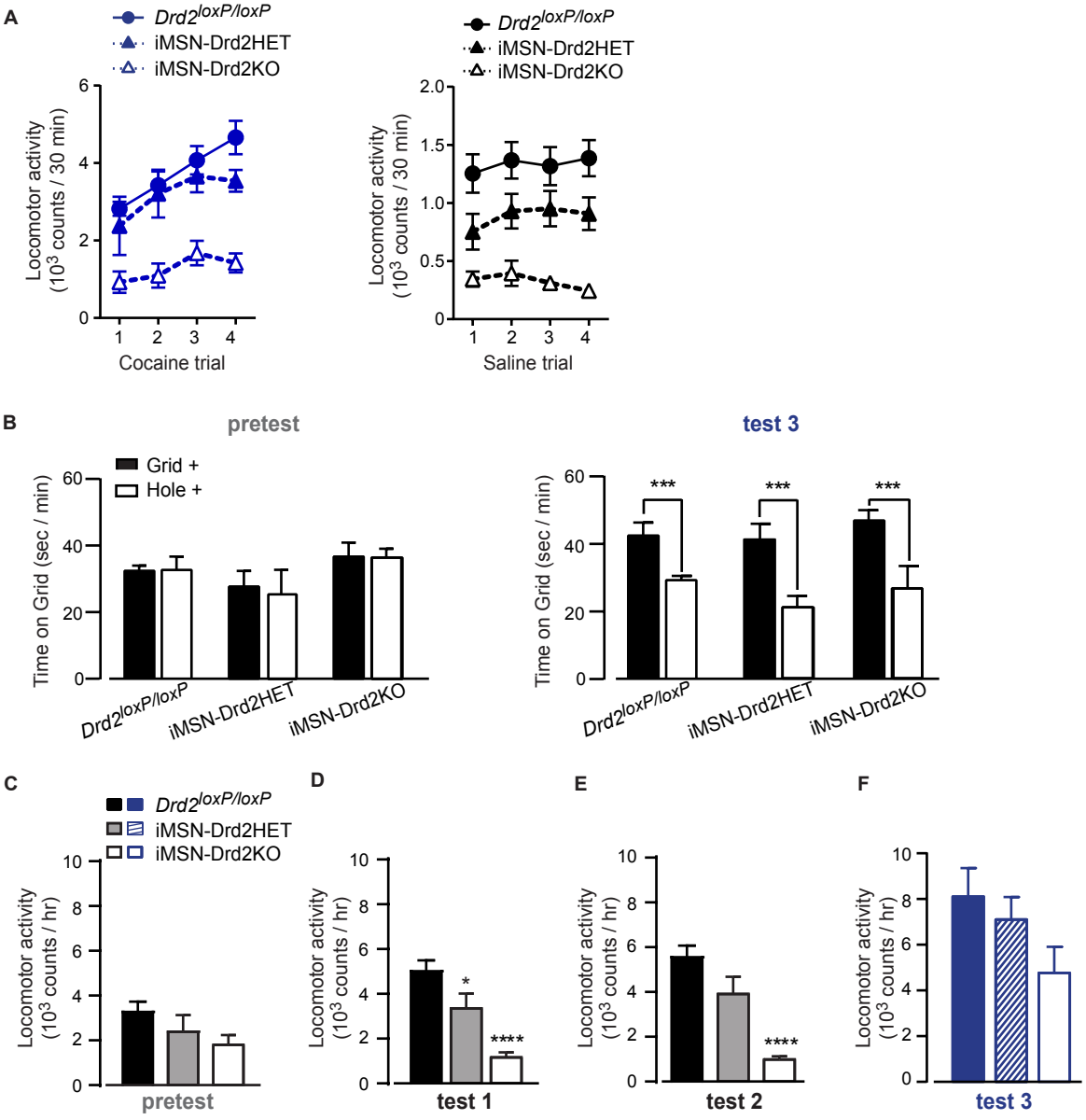

Figure S5, related to Figure 6: iMSN-Drd2KO mice acquire cocaine place preference. A, Locomotor activity during cocaine (left, 15 mg/kg) and saline (right) conditioning trials in *Drd2*$^{loxP/loxP}$ (n = 11), iMSN-Drd2HET (n = 7), and iMSN-Drd2KO (n = 11) mice (3WRM ANOVA; genotype: $F_{2,25}$ = 30.31, $p < 0.0001$; trial type x genotype: $F_{2,25}$ = 10.89, $p < 0.0001$; post-hoc: p's < 0.01 iMSN-Drd2KO vs. iMSN-Drd2HET and *Drd2*$^{loxP/loxP}$ for cocaine and saline trials). **B**, Time on grid floor for the cocaine conditioning groups Grid+ (filled) and Hole+ (open) at pretest (left) and test 3 (right) (2WANOVA; conditioning: $F_{1,23}$ = 27.95, $p < 0.0001$). **C-F**, Locomotor activity for the saline-primed pretest (**C**), test 1 (**D**) and test 2 (**E**), and the cocaine-primed (15 mg/kg) test 3 (**F**). Post-hoc: **** $p < 0.0001$ vs. *Drd2*$^{loxP/loxP}$. All data expressed as mean ± s.e.m.

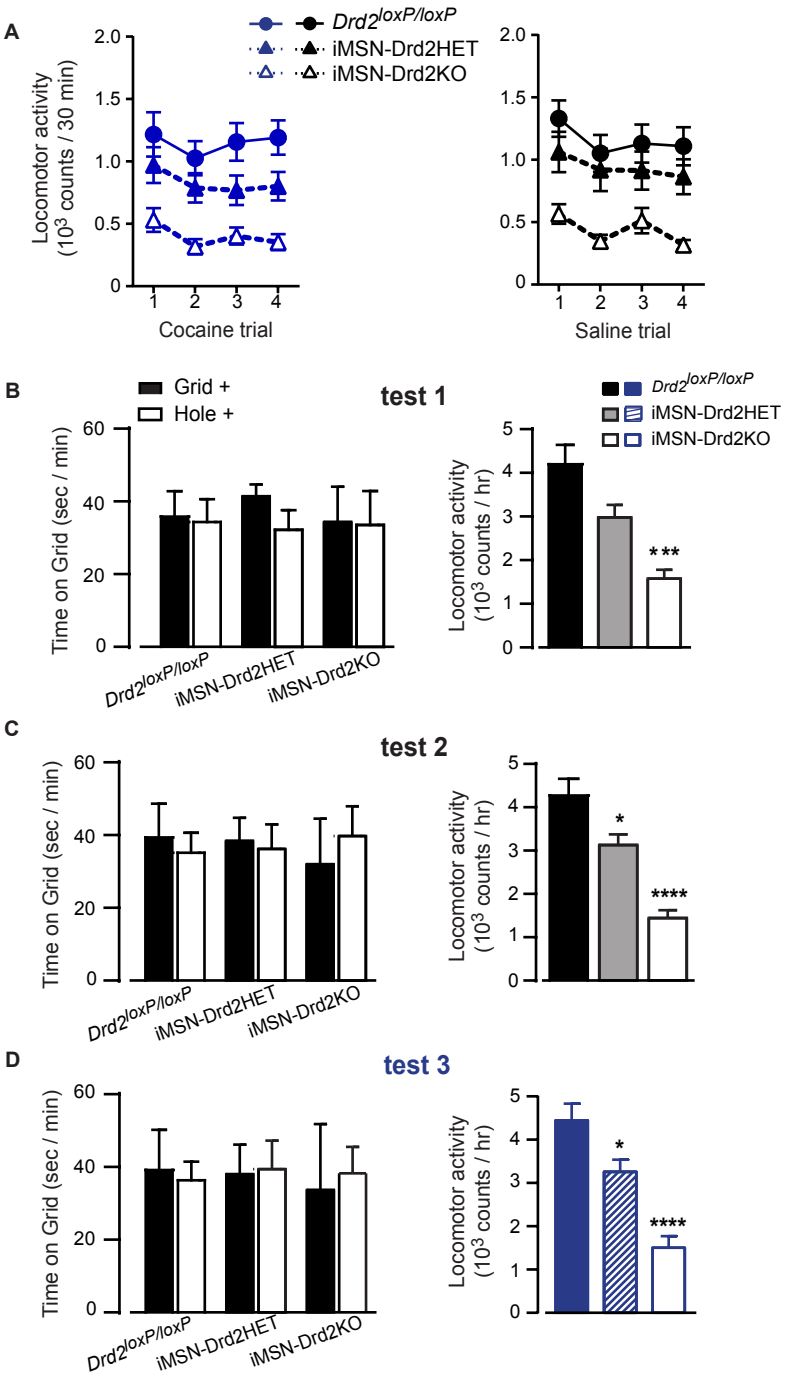

Figure S6, related to Figure 6: iMSN-Drd2KO mice do not acquire a low-dose cocaine preference. A, Locomotor activity during cocaine (left, 0.5 mg/kg) and saline (right) conditioning trials for *Drd2*$^{loxP/loxP}$ (n = 12), iMSN-Drd2HET (n = 10), and iMSN-Drd2KO (n = 7) mice. **B-D**, Time on grid floor for the cocaine conditioning groups Grid+ (filled) and Hole+ (open) (left) and locomotor activity (right) for the saline primed test 1 (**B**) and test 2 (**C**), and cocaine-primed (0.5 mg/kg) test 3 (**D**). Post-hoc: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ vs. *Drd2*$^{loxP/loxP}$. All data expressed as mean ± s.e.m.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals: iMSN-Drd2HET mice were generated by crossing heterozygous *Drd2*$^{wt/loxP}$ mice with heterozygote *Adora2a-Cre* mice. *Adora2a-Cre* mice crossed with Drd1a-td-Tomato mice (Tg(Drd1a-tdTomato)6Calak, JAX016204) (Ade et al., 2011) were used to quantify the extent of co-expression of the D1R and D2R in iMSNs in the NAc.

Viral Vectors: Viral vectors with Cre-dependent expression of ChR2, G_i-DREADD hM4Di, mCherry were used. To knockout D2Rs in adulthood a Cre expressing vector or a control vector expressing EGFP was used.The ChR2 vectors used were AAV5-EF1a-DIO-ChR2(H134R)-mCherry, ($3x10^{12}$, UNC) or rAAV5-EF1a-DIO-hChR2(H134R)-EYFP ($4.5x10^{12}$, UNC). The control mCherry vector was AAV5-hSyn-DIO-mCherry (3-6 $x10^{12}$, UNC). The Gi-DREADD hM4Di vector used was AAV5-hSyn-DIO-hM4Di-mCherry (1-6 $x10^{13}$, UNC). The Cre viral vector used was AAV9-CMV-HI.eGFP-Cre.SV40 ($6.5x10^{12}$, Penn Vector Core AV9-PV2004) and the control vector expressing EGFP was (AAV9-CB7-CI-EGFP-RBG, $2.05x10^{13}$, Penn Vector Core). AAV5.CAG.Flex.eGFP.WPRE.bGH was used to express GFP in the co-localization analysis. Viral vector infection efficiency was 80 ± 3 % (68 – 96 %).

Genotyping: Primers used for PCR of the *Drd2*^{loxP} allele (5′-GCT TCA CAG TGT GCT GCC TA-3′ and 5′-CCA TTG CTG CCT CTA CCA AG-3′) and the *Adora2a-Cre* transgene (5'-CGT GAG AAA GCC TTT GGG AAG CT-3' and 5' CGG CAA ACG GAC AGA AGC ATT 3') were obtained from Integrated DNA Technologies and protocol followed as recommended by strain provider.

Co-localization analysis of markers for D1R- and D2R-expressing MSNs: There is a population of MSNs that co-expresses D1 and D2 receptors, and we performed analysis to estimate the extent of D1R expression in iMSNs. Double transgenic mice, *Adora2a-Cre*;Drd1-tdTomato, were stereotaxically injected in the NAc core with a vector expressing eGFP in a Cre-dependent fashion (as previously done for ChR2 and hM4Di expression). The co-localization of td-Tomato, a marker for D1R-expressing dMSNs, with eGFP, a marker for D2R-expressing iMSNs, was quantified using ImageJ software in images acquired with a confocal microscope. We found that 4.8% of all MSNs were co-labeled for markers of D2R- and D1R-expressing neurons and 9.5% of iMSNs were co-labeled with tdTomato. Similar percentages were found when these same experiments were performed using iMSN-Drd2KO instead of *Adora2a-Cre* mice (4.2% no difference between genotypes: unpaired t-test, $p > 0.05$). These results indicate that when using the *Adora2a-Cre* mouse line to target iMSNs there is a third small population of neurons that co-labels for markers of both iMSNs and dMSNs, and it corresponds to 4-5% of all MSNs in the NAc. The degree of co-expression found here is in agreement with previous reports for co-localization of D1Rs and D2Rs in MSNs of adult animals (Bertran-Gonzalez et al., 2008; Gangarossa et al., 2013; Matamales et al., 2009; Shuen et al., 2008; Thibault et al., 2013).

Locomotor activity: Sample size determination was based on previous data from our laboratory, which show that a sample size of 8 subjects per treatment group is sufficient for the detection of between-group differences. Naïve mice (8-20 weeks old) were used for all experiments. Locomotor activity was measured during the animal's light cycle for 1 hour in a clear polycarbonate chamber (20 cm H x 17 cm W x 28 cm D) equipped with infrared photobeam detectors (Columbus Instruments). Locomotor activity was measured for 1 h unless otherwise stated.

Cocaine dose-response: As expected, repeated cocaine administration resulted in a sensitized locomotor response to the 25 mg/kg cocaine dose (Main effect of dosing: $F_{1,25} = 6.328$, $p = 0.0187$); however, the magnitude of the sensitized response was not different between genotypes (Main effect of genotype: $p = 0.9$; Genotype x dosing interaction: $p = 0.76$). Therefore, the data from repeated and single administrations were collapsed for each genotype.

Intra-VP agonist locomotor response: The locomotor chamber was constructed in house out of clear polycarbonate (33 x 54 x 20 cm, H x W x D) and housed in a sound and light attenuating enclosure. Microinjectors were tethered to a single channel fluid swivel on a counterbalanced arm (Instech) that was mounted on the locomotor chamber. Mice were gently restrained and a bilateral microinjector was inserted into the cannula that extended into the VP. Bilateral microinjectors were secured to the cannula pedestal by a Tygon tubing collar and connected via CoEx PE/PVC tubing (.017/.4 mm ID x .051/1.3 mm OD) to the syringe on a pump (World Precision Instruments) located outside of the sound-attenuating enclosure. Locomotion was recorded via a ceiling mounted video camera (Q-See) and analyzed off-line with Ethovision XT9 (Noldus).

Conditioned place preference: Unbiased conditioning chambers (33 x 54 x 20 cm, H x W x D, clear polycarbonate) were separated into two equal compartments by a clear divider and housed in sound- and light-attenuating cabinets

equipped with a ventilation fan (Applying Reason and Technology LLC, Gaithersburg, MD). Subject's side position and locomotor activity was detected with an infrared video camera (Q-See) and infrared photobeam detectors (Columbus Instruments), respectively. The enclosure was not illuminated during conditioning or testing. The conditioned stimuli were tactile, stainless steel grid (1.9 mm diameter woven rods) and hole (4.7 mm holes, 1.6 mm apart) floors. Mice were randomized to receive cocaine paired with the grid (Grid+) or hole (Hole+) floors, and the first conditioning day was counterbalanced for drug. Place preference was analyzed using Ethovision XT9 (Noldus).

Fast scanning cyclic voltammetry: Data were collected with a retrofit headstage (CB-7B/EC with 5 MΩ resistor) using a Multiclamp 700B amplifier after low-pass filter at 10 kHz and digitized at 100 kHz using pClamp10 software (all from Molecular Devices). Voltammetric analysis was done using custom-written software in Igor Pro software. The custom software is freely available upon request. The voltamogram, peak amplitude, and area of the DA transient were measured. Experiments were rejected when the evoked current did not have the characteristic electrochemical signature of DA assessed by a current-voltage plot. The cocaine concentration used is within the range found in the brain following administration of cocaine doses that evoke locomotor activation in mice (Zombeck et al., 2009) and within the range of concentrations (0.01 – 60 μM) for inhibition of the DA transporter within the NAc and caudate (Jones et al., 1995).

Drugs: Cocaine HCl was obtained from the National Institute on Drug Abuse. NBQX, (R)-CPP, SKF 81297, and CGP were from Tocris; quinpirole, QX314, picrotoxin, and CNO were from Sigma; (R)-baclofen and gabazine (SR95531) were from Abcam. All i.p. injections were administered at 10 ml/kg.

SUPPLEMENTAL REFERENCES

Ade, K.K., Wan, Y., Chen, M., Gloss, B., and Calakos, N. (2011). An Improved BAC Transgenic Fluorescent Reporter Line for Sensitive and Specific Identification of Striatonigral Medium Spiny Neurons. Front Syst Neurosci 5, 32.

Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamales, M., Herve, D., Valjent, E., and Girault, J.A. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci 28, 5671-5685.

Gangarossa, G., Espallergues, J., Mailly, P., De Bundel, D., de Kerchove d'Exaerde, A., Herve, D., Girault, J.A., Valjent, E., and Krieger, P. (2013). Spatial distribution of D1R- and D2R-expressing medium-sized spiny neurons differs along the rostro-caudal axis of the mouse dorsal striatum. Front Neural Circuits 7, 124.

Jones, S.R., Garris, P.A., and Wightman, R.M. (1995). Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. The Journal of pharmacology and experimental therapeutics 274, 396-403.

Matamales, M., Bertran-Gonzalez, J., Salomon, L., Degos, B., Deniau, J.M., Valjent, E., Herve, D., and Girault, J.A. (2009). Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. PloS one 4, e4770.

Shuen, J.A., Chen, M., Gloss, B., and Calakos, N. (2008). Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. J Neurosci 28, 2681-2685.

Thibault, D., Loustalot, F., Fortin, G.M., Bourque, M.J., and Trudeau, L.E. (2013). Evaluation of D1 and D2 dopamine receptor segregation in the developing striatum using BAC transgenic mice. PloS one 8, e67219.

Zombeck, J.A., Gupta, T., and Rhodes, J.S. (2009). Evaluation of a pharmacokinetic hypothesis for reduced locomotor stimulation from methamphetamine and cocaine in adolescent versus adult male C57BL/6J mice. Psychopharmacology 201, 589-599.