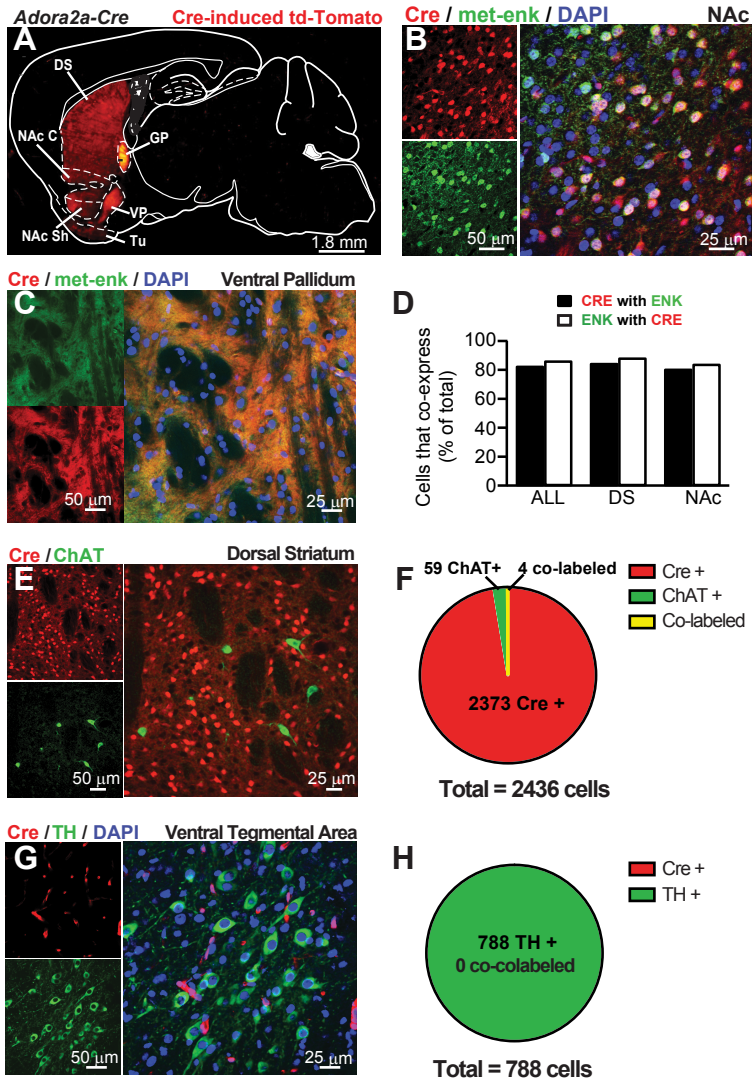

Figure S1, relates to Figure 1. Pattern of cre-dependent recombination in *Adora2A-Cre*$^{+/-}$ mice. a) Representative low-power fluorescent image of sagittal brain section showing pattern of cre-dependent recombination in *Adora2A-Cre*$^{+/-}$ mice. **b,c**) Representative images showing colocalization of met-enkephalin immunoreactivity (green, met-enk), cre (red), and DAPI (blue) in the NAc (b) and ventral pallidum (c). **d**) Proportion of colocalization of cre with met-enkephalin and met-enkephalin with cre in the striatum (n = 2782-2902 cells/3 mice). **e**) Representative image of choline acetyltransferase immunoreactivity (ChAT; green) and cre (red) colocalization in the dorsal striatum. **f**) Proportion of cre, ChAT, and co-labeled cells (n = 4/2436 cells/3 mice) **g**) Representative image of tyrosine hydroxylase immunoreactivity (TH; green), cre (red) and DAPI (blue) colocalization in the ventral tegmental area. **h**) Proportion of Cre, TH and co-labeled cells in the ventral tegmental area (n = 0/788 cells/3 mice).

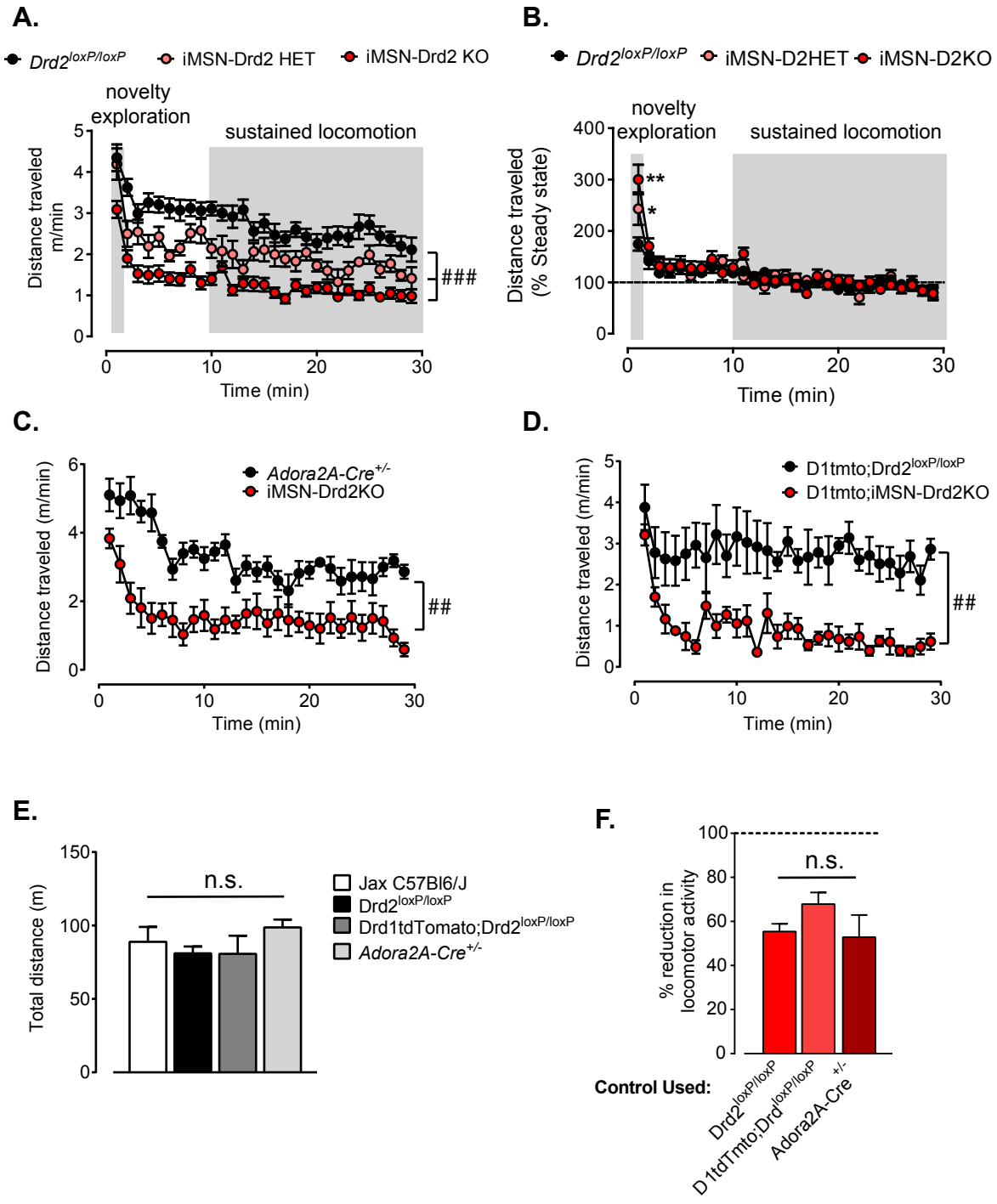

Figure S2, relates to Figure 1. Timecourse of locomotor activity in a novel open field for control and iMSN-Drd2KO mice. a) Timecourse of locomotor activity in 40 x 40 cm novel open field *Drd2*$^{loxP/loxP}$, iMSN-Drd2HET and iMSN-Drd2KO (two-way RM ANOVA, main effect of genotype, $F_{2,51} = 34.36$, $p > 0.001$, n = 9-28). **b**) Locomotor activity normalized to "sustained phase" activity for *Drd2*$^{loxP/loxP}$, iMSN-Drd2HET and iMSN-Drd2KO (% of steady state x genotype interaction, $F_{56, 1400} = 1.86$, $p < 0.001$, n = 9-28). *, ** post-hoc tests, $p < 0.05$, 0.01 respectively. **c**) Timecourse of locomotor activity of non-littermate *Adora2A-Cre*$^{+/-}$ and iMSN-Drd2KO mice (two-way RM ANOVA, main effect of genotype, $F_{1,10} = 20.52$, $p < 0.01$, n = 6 each). **d**) Timecourse of locomotor activity of *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice crossed onto a *Drd1tdTomato* background, (two-way RM ANOVA, main effect of genotype, $F_{1,196} = 14.30$, $p < 0.01$, n = 4-5). **d**) Total distance traveled for WT JAX C57Bl6/J mice and control mice used for the study: *Drd2*$^{loxP/loxP}$, *Drd1tdTomato;Drd2*$^{loxP/loxP}$, *Adora2A-Cre*$^{+/-}$ (one-way ANOVA, $F_{3,34} = 1.156$, $p > 0.05$, n = 5-21). **e**) There was no difference in the % reduction in locomotor activity of iMSN-Drd2KO or *Drd1tdTomato;iMSN-Drd2KO* mice relative to the average distance traveled of each of the appropriate control groups.

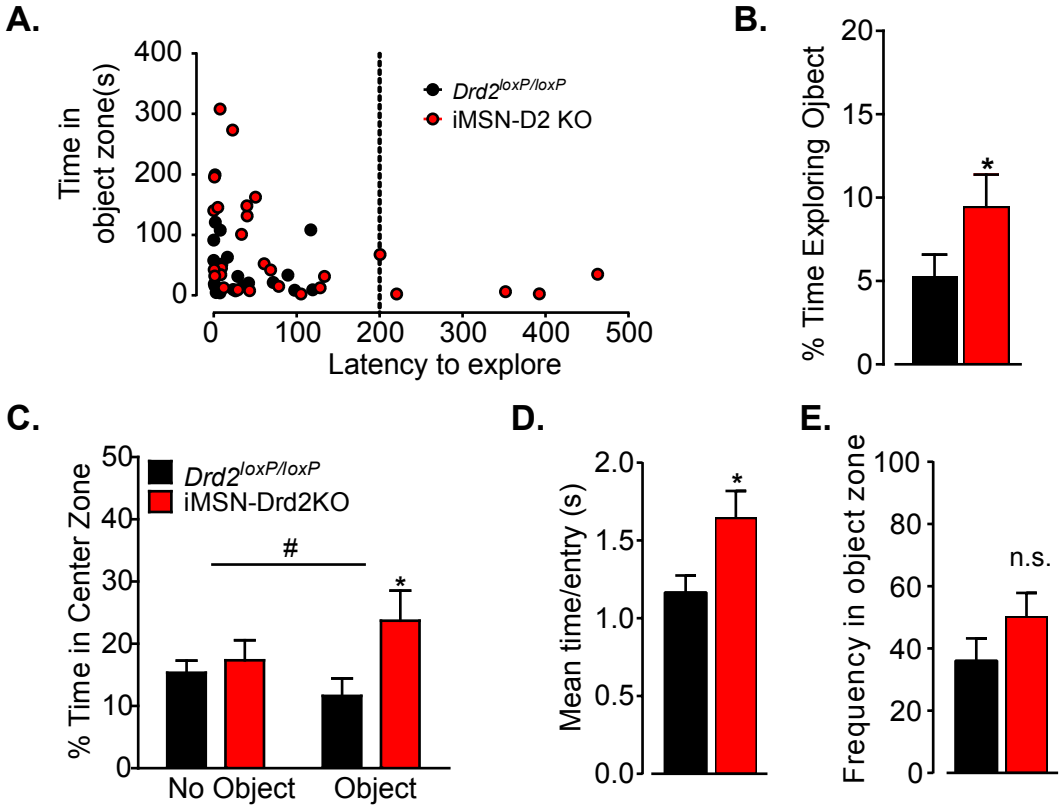

Figure S3, relates to Figure 1. Additional novel object exploration data. **a**) Time in object zone x latency to explore scatter plot. A small subset (5 animals) of iMSN-Drd2KO mice had ≥ 200s latencies to explore the novel object (20% of the session time) and were excluded from the majority of the analysis. **b**) When including all the mice, including iMNS-Drd2KO mice with long latencies, there was still a significant increase in % time exploring the object relative to the time elapsed following the initial investigation (total session time – latency to explore) (one-tailed t-test, $p < 0.05$, n = 21-25). **c**) Percent time in center without and with a novel object present for *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice (% time in center x genotype interaction, two-way RM ANOVA, $F_{1,39} = 4.13$, $p < 0.05$, post-hoc t-test on "object", $p < 0.05$, n = 20-21) **d**) Mean time/object zone entry for *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice (t-test, $p < 0.05$, n = 20-21). **e**) Frequency to enter the object zone for *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice (t-test, $p > 0.05$, n = 20-21).

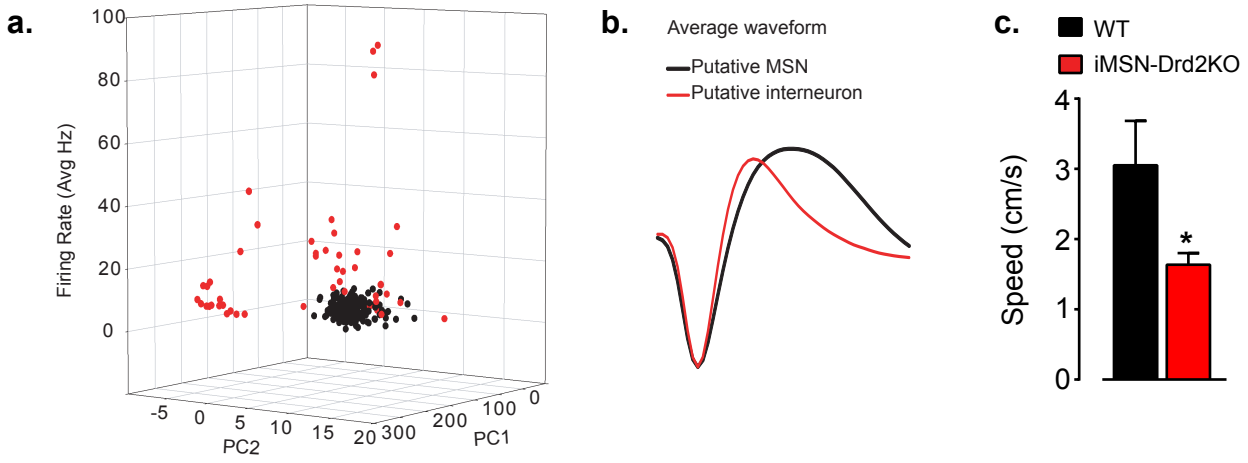

Figure S4, relates to Figure 4. Sorting criteria for single units deriving from medium spiny neurons versus interneurons. a) Graphical projection of clustering strategy for identifying putative medium spiny neurons (MSNs) and interneurons, based on principal component analysis of the waveform shape (PC1, PC2), as well as firing rate. Putative MSNs formed a dense cluster of low firing neurons with similar waveforms (black dots). Putative interneurons demonstrated more diversity in both firing rate and waveform shape (red dots). **b)** Average waveform of putative MSNs (black) and interneurons (red). **c)** Speed of control and iMSN-Drd2KO mice measured while recording single unit activity *in vivo* (one-tailed t-test, $p < 0.05$, n = 5-7).

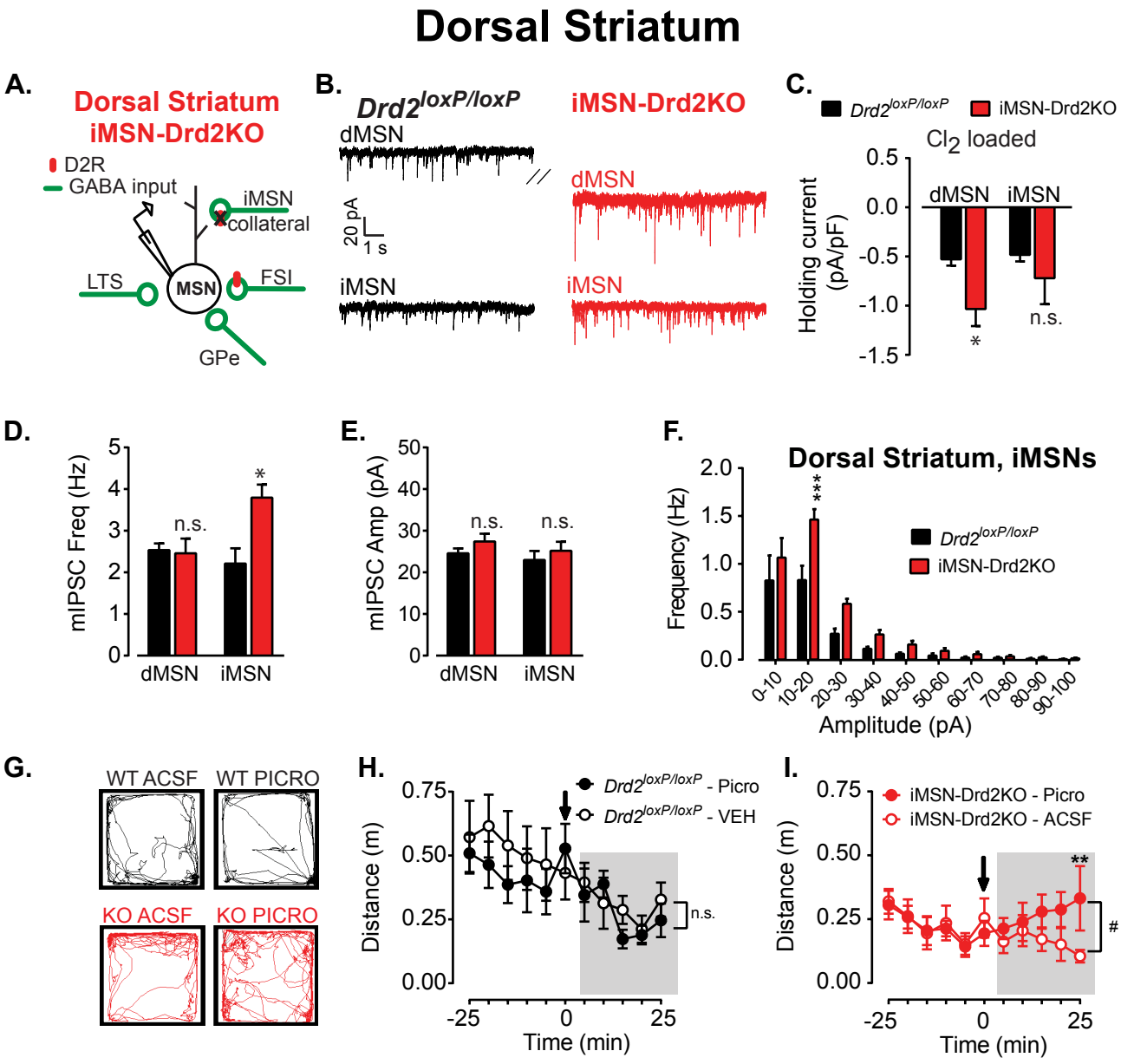

Figure S5, relates to Figure 6 & 7. Enhanced GABA transmission in the dorsal striatum (DS) of iMSN-Drd2KO mice. **a**) Schematic depicting the main sources of synaptic GABAergic inputs (green) onto MSNs in the DS. **b**) Example traces of mIPSCs recorded from putative dMSNs and iMSNs in the DS of *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice. Hatch mark indicates a significant difference in holding current between dMSNs recorded in control and KO mice. **c**) Increase in holding current density in DS dMSNs of iMSN-Drd2KO mice compared to *Drd2*$^{loxP/loxP}$ (t-test, $p < 0.001$, n = 11 per group), but not DS iMSNs (t-test, $p > 0.05$, n = 7-8). **d**) Increase in mIPSC frequency in DS iMSNs of iMSN-Drd2KO mice compared to *Drd2*$^{loxP/loxP}$ (t-test, $p < 0.01$, n = 7-8), but not DS dMSNs (t-test, $p > 0.05$, n = 11 per group). **e**) No difference in mIPSC amplitude between genotypes for either cell type (t-test, $p > 0.05$, n = 7-11). **f**) Frequency histogram of mIPSC amplitude for DS iMSNs for both genotypes. **g**) Representative track traces for *Drd2*$^{loxP/loxP}$ (black) and iMSN-Drd2KO (red) mice following vehicle (ACSF) (left) or picrotoxin (100 µM) (right) infusion into the DS (AP +0.5, ML ± 1.5, DV 3.0 mm). **h**) Timecourse of locomotor activity in *Drd2*$^{loxP/loxP}$ mice before and after infusion of vehicle (ACSF) (open circle) or picrotoxin (closed circles), n = 8. **i**) Timecourse of locomotor activity in iMSN-Drd2KO mice before and after infusion of vehicle (ACSF) (open circle) or picrotoxin (closed circles), n = 9. # time x drug interaction, $p < 0.05$, ** post-hoc tests, $ps < 0.01$.

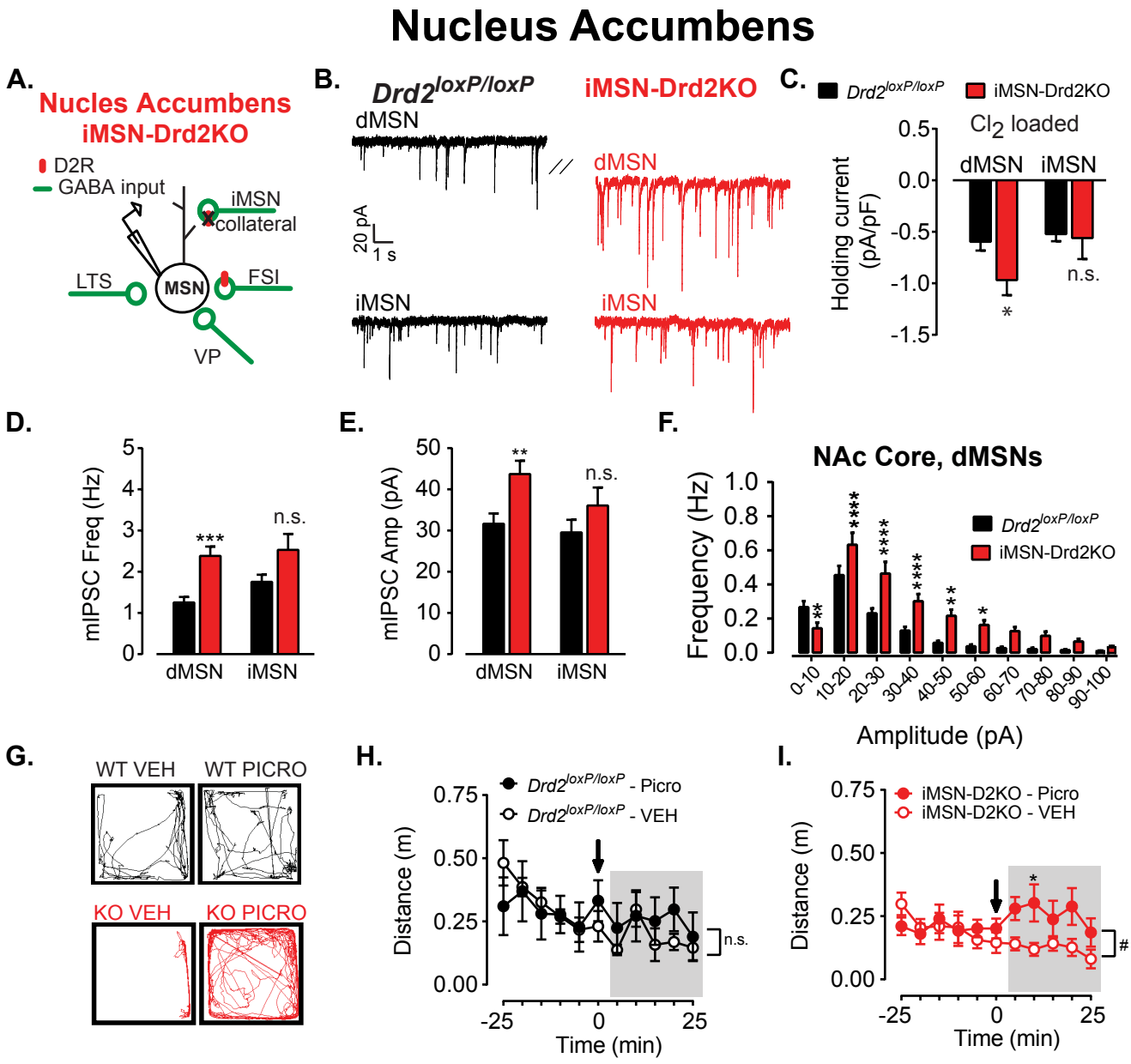

Figure S6, relates to Figure 6 & 7. Enhanced GABA transmission selectively in dMSNs in the nucleus accumbens (NAc) of iMSN-Drd2KO mice. **a)** Schematic depicting the main sources of synaptic GABAergic inputs (green) onto MSNs in the NAc. **b)** Example traces of mIPSCs recorded from putative dMSNs and iMSNs in the NAc of *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice. Hatch mark indicates a significant difference in holding current between dMSNs recorded in control and KO mice. **c)** Increase in holding current density in NAc dMSNs of iMSN-Drd2KO mice compared to *Drd2*$^{loxP/loxP}$ (t-test, $p < 0.05$, n = 13-14), but not NAc iMSNs (t-test, $p > 0.05$, n = 7 per group). **d)** Increase in mIPSC frequency in NAc dMSNs of iMSN-Drd2KO mice compared to *Drd2*$^{loxP/loxP}$ (t-test, $p < 0.001$, n = 13-14), but not NAc iMSNs (t-test, $p > 0.05$, n = 7 per group). **e)** Increase in mIPSC amplitude in NAc dMSNs of iMSN-Drd2KO mice compared to *Drd2*$^{loxP/loxP}$ (t-test, $p < 0.01$, n = 13-14), but not NAc iMSNs (t-test, $p > 0.05$, n = 7 per group). **f)** Frequency histogram of mIPSC amplitude NAc dMSNs for both genotypes. **g)** Representative track traces for *Drd2*$^{loxP/loxP}$ (black) and iMSN-Drd2KO (red) mice following vehicle (ACSF) (left) or picrotoxin (100 µM) (right) infusion into the NAc (AP +1.1, ML ± 1.0, DV 4.75 mm). **h)** Timecourse of locomotor activity in *Drd2*$^{loxP/loxP}$ mice before and after infusion of vehicle (ACSF) (open circle) or picrotoxin (closed circles), n = 7. **i)** Timecourse of locomotor activity in iMSN-Drd2KO mice before and after infusion of vehicle (ACSF) (open circle) or picrotoxin (closed circles), n = 7. # time x drug interaction, $p < 0.05$, * post-hoc tests, $p < 0.05$.

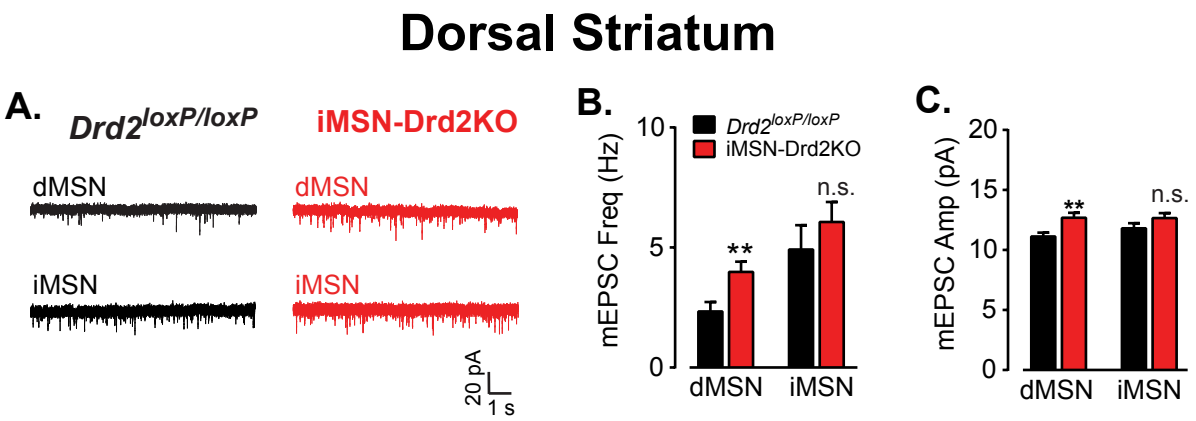

Figure S7, relates to Figure 6. Alteration in glutamatergic transmission selectively in dMSNs recorded in the DS of iMSN-Drd2KO mice. **a**) Example traces of mEPSCs recorded from putative dMSNs and iMSNs in the DS of *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice. **b**) Increase in mEPSC frequency in DS dMSNs of iMSN-Drd2KO mice compared to *Drd2*$^{loxP/loxP}$ (t-test, $p < 0.01$, n = 14-16), but not DS iMSNs (t-test, $p > 0.05$, n = 12-15). iMSNs had a higher mEPSC frequency compared to dMSNs irrespective of genotype (main effect of cell type, $p < 0.01$). **c**) Increase in mEPSC amplitude in DS dMSNs of iMSN-Drd2KO mice compared to *Drd2*$^{loxP/loxP}$ (t-test, $p < 0.01$, n = 14-16), but not DS iMSNs (t-test, $p > 0.05$, n = 12-15).

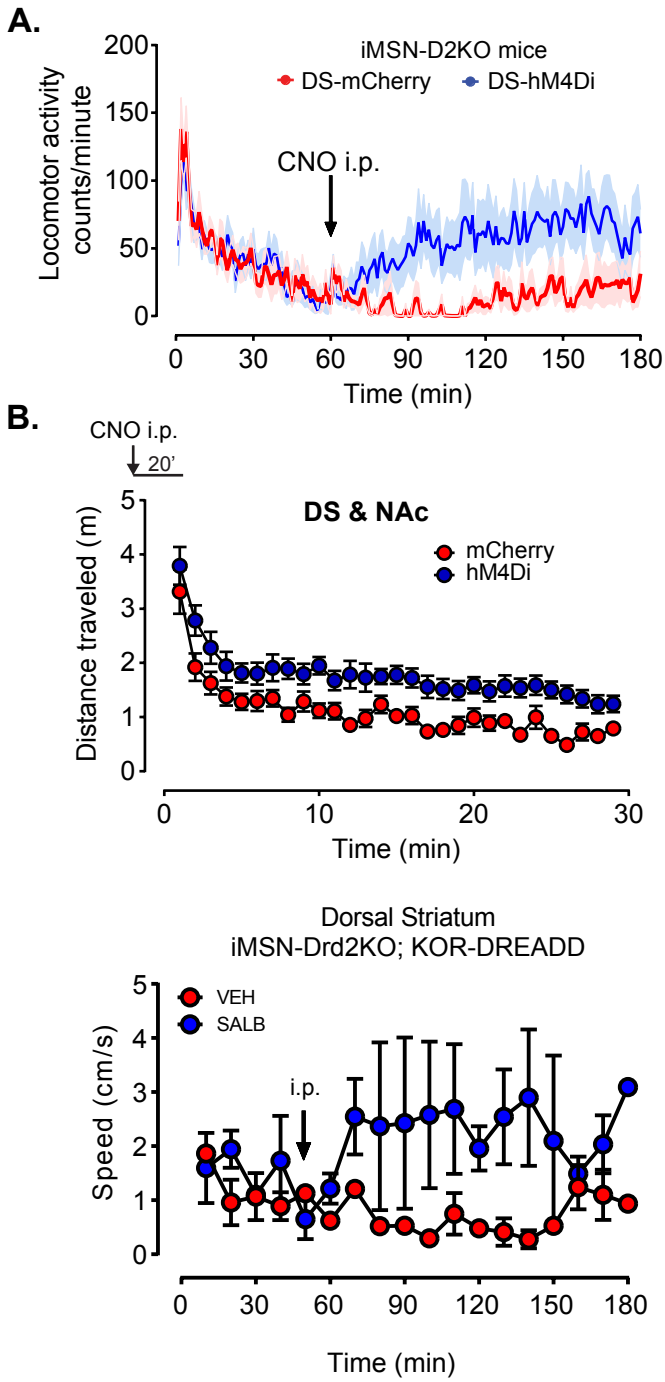

Figure S8, relates to Figure 8. Gi-DREADD mediated rescue of locomotor activity. **a**) Locomotor activity (counts/min) over a 3hr session in which iMSN-Drd2KO animals expressing hM4Di-mCherry or mCherry control viral vector in iMSNs in the DS were habituated for 1 hr, then given CNO and run for an additional 2hrs. Following CNO injection, hM4Di-mCherry expressing mice moved significant more (time x genotype interaction, two-way RM ANOVA, $F_{179,2864} = 2.20$, $p < 0.001$, n = 8 -11). **b**) Timecourse of locomotor activity (meter/min) in a novel open field for iMSN-Drd2KO mice expressing hM4Di-mCherry or mCherry control viral vector (combined data from NAc and DS injected animals). Both groups were given CNO (1mg/kg, *i.p.*) 30 minutes prior to a 30 minute novel open field session (main effect of genotype, two-way RM ANOVA, $F_{1,33} = 11.55$, $p < 0.001$, n = 16-21). **c**) Average speed following either vehicle or salvinorinB (17 mg/kg i.p.) injection in iMSN-Drd2KO animals expressing KOR-DREADD in iMSNs in the DS. These mice correspond to the mice used for the *in vivo* GPe recordings shown in Figure 9. There was a statistical trend for a main effect of drug (main effect of genotype, two-way RM ANOVA, $F_{17,17} = 1.77$, $p = 0.12$, n = 2).

Full Experimental Procedures:

All procedures were performed in accordance with guidelines from the National Institute on Alcohol Abuse and Alcoholism's or the National Institute for Diabetes and Digestive and Kidney Diseases' Animal Care and Use Committee.

Animals: Mice (p60-180) were group housed unless otherwise specified and kept under a 12h:12h light cycle (6:30 ON/18:30 OFF) with food and water available *ad libitum*. There was no evidence of sex differences in our behavioral measurements and therefore both males and females were used and data from males and females were combined. iMSN-D2KO (*Drd2*$^{loxP/loxP}$; *Adora2A-Cre*$^{+/-}$) mice were generated by crossing *Drd2*$^{loxP/loxP}$ mice which carry the conditional *Drd2* alleles in homozygosity, with *Adora2a-Cre*$^{+/-}$ mice, which express Cre recombinase in heterozygosity under the adenosine 2a receptor promoter. The offspring were approximately 50% iMSN-D2KO (*Drd2*$^{loxP/loxP}$;*Adora2A-Cre*$^{+/-}$) and 50% control littermates *Drd2*$^{loxP/loxP}$ mice. iMSN-D2HET mice were generated by crossing heterozygous *Drd2*$^{wt/loxP}$ mice with *Adora2a-Cre*$^{+/-}$ mice, which produced 50% iMSN-D2HET mice and 50% control littermates *Drd2*$^{wt/loxP}$. There was a similar distribution of male and female mice for control, iMSN-Drd2HET and iMSN-Drd2KO groups for all experiments. In order to identify and target dMSNs for recordings, *Drd2*$^{loxP/loxP}$ and iMSN-D2KO mice were crossed onto a *Drd1a-tdTomato* background. Breedings were set up to yield *Drd1a-tdTomato*$^{+-}$;*Drd2*$^{loxP/loxP}$ and *Drd1a-tdTomato*$^{+/-}$;iMSN-Drd2KO littermates. For experiments requiring ChR2 expressing in control mice, non-littermate *Adora2A-Cre*$^{+/-}$ mice were used. Note that we confirmed that there is no difference in the locomotor activity of *Adora2A-Cre*$^{+/-}$ and *Drd2*$^{loxP/loxP}$ mice.

Commercially available mouse lines used in this study

Catalog name	Catalog #	Abbreviation	Source	Citation
B6.129S4(FVB)-*Drd2*$^{tm1.1Mrub}$/J	JAX020631	*Drd2*$^{loxP/loxP}$	Jax; M. Rubinstein	(Bello et al., 2011)
B6.FVB(Cg)-Tg(*Adora2a-cre*)KG139Gsat/Mmucd,	036158-UCD	*Adora2a-Cre*$^{+/-}$	MMRC; C. Gerfen	(Gerfen et al., 2013)
B6.C6-Tg(Drd1a-tdTomato)6Calak/J	JAX016204	*Drd1a-tdTomato*	Jax; N. Calakos	(Shuen et al., 2008)

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′), the *Adora2a-Cre* transgene (5'-CGT GAG AAA GCC TTT GGG AAG CT-3' and 5' CGG CAA ACG GAC AGA AGC ATT 3') and *Drd1a-tdTomato* (5'-CTT CTG AGG CGG AAA GAA CC-3' and 5'-TTT CTG ATT GAG AGC ATT CG-3') were obtained from Integrated DNA Technologies and protocol followed as recommended by strain provider. We outsourced some of our genotyping to Transnetyx, Inc (Cardova, TN) who conducted qPCR on our samples using the same primer sequences.

Quantitative polymerase chain reaction: Total RNA was purified using RNeasy Micro (Qiagen), and cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad). *Actb* (Mm01205647) and *Drd2* (Mm00438541_m1) TaqMan Gene Expression Assays (Applied Biosystems) were used to determine relative mRNA expression of the endogenous control gene β-actin and DA D2Rs, respectively. Quantitative PCR (qPCR) was performed using TaqMan Fast Polymerase (Applied Biosystems) in a StepOnePlus Real-Time PCR system. Cycling conditions were as follows: initial hold at 95°C for 20 s; 40 cycles of step 1 (95°C for 1 s); and step 2 (60°C for 20 s). Samples were run in quadruplicates, and negative controls were run in parallel. cDNA synthesis and qPCR experiments were repeated three times. Relative quantification was calculated using the ΔΔCt method (StepOne System Software, Applied Biosystems).

Stereotaxic viral vector injection: Mice (8-12 week old) were anesthetized with isoflurane (2% in O_2) and placed in stereotaxic frame. Mice that underwent surgery were individually housed following the surgery and remained so throughout experimentation. Mice that underwent surgery were individually housed following the surgery and remained so throughout experimentation. Injections were performed at low speed (100 nl/min) and injection needle was kept in place for at least 4 minutes following the infusion to promote proper diffusion and prevent backflow. All

experiments were performed at least 3-4 weeks after surgery. Viral expression was confirmed by fluorescence visualization.

Viral vectors and stereotaxic coordinates

Viral Vector	Abbreviation	Serotype/Titer	Volume /side	Injections coordinates	Mouse line	Experiment	Source
AAV-CMV-hGHintron.GFP-Cre.SV40	Cre	serotype 2/9, titer = $6.5x10^{12}$	300 nL	AP, +1.1; ML, ±1.0; DV, 4.5 mm	*Drd2*$^{loxP/loxP}$	NAc-Drd2 knock-down	Penn Vector Core
AAV-CB7-CI-EGFP-RBG	GFP	serotype 2/9, titer = $2.05x10^{13}$	300 nL	AP, +1.1; ML, ±1.0; DV, 4.5 mm	*Drd2*$^{loxP/loxP}$	Control vector for NAc-Drd2 KD exp	Penn Vector Core
AAV/hSyn-DIO-hM4Di-mCherry	hM4Di or Gi-DREADD	serotype 2/5, titer = 3-6 $x10^{12}$	300 nL (NAc) 600 nL (DS)	NAc: AP, +1.1; ML, ±1.0; DV, 4.5 mm DS: AP, +0.5; ML, ±1.5; DV, 2.7 and 3.3 mm	iMSN-Drd2KO	-tonic GABA current rescue -locomotor rescue	UNC vector core
rAAV/hSyn-DIO-mCherry	mCherry	serotype 2/2, titer = 3-6 $x10^{12}$	300 nL (NAc) 600 nL (DS)	NAc: AP, +1.1; ML, ±1.0; DV, 4.5 mm DS: AP, +0.5; ML, ±1.5; DV, 2.7 and 3.3 mm	iMSN-Drd2KO	Control vector for hM4Di locomtor experiments	UNC vector core
rAAV-Ef1a-DIO-hChR2(H134R)	ChR2	serotype 8, titer = of $8x10^{12}$	1.5 µL for *in vivo*	DS: A/P: +0.8, Lat: +1.5, D/V: -2.7 mm from top of skull NAc: AP, +1.1; ML, ±1.0; DV, 4.5 mm	*Adora2A-Cre*$^{+/-}$ or iMSN-Drd2KO	Optogenetic tagging during *in vivo* recording Optogenetic evoked IPSC	Penn Vector Core
Syn-DIO-hKORD-IRES-mCit-WPRE	KOR-DREADD or Gi-DREADD	serotype 8, titer=$1.2x10^{13}$	500 nL	A/P: +0.8, Lat: +1.5, D/V: -2.7 mm from top of skull	iMSN-Drd2KO	*In vivo* recordings from GPe of iMSN-Drd2KO	UNC Vector Core

Bilateral Guide Cannula Implantation: Bilateral cannula were implanted in the DS (AP, +0.5; ML, ±1.5; DV, 2.0mm; injector had 1mm protrusion past guide, final DV, 3.0 mm) or (NAc AP, +1.1; ML, ±1.0; DV, 3.0 mm, injector had 1.75 mm protrusion past guide, final DV, 4.5 mm) (Plastics One). Metabond (Parkel, Inc) was applied prior to application of the dental cement to secure the headcap to the skull. (Plastics One). Animals that were only implanted with guide cannula were given at least 10 days to recover from surgery. Animals given both viral injections and guide cannulae were not used for behavior until approximately 4 weeks after surgery. Histology was performed to confirm correct cannula placements.

Behavior: ***Locomotor activity in homecage****:* Mice were housed individually many weeks prior to experimentation. Prior to the start of the experiment, mice were kept in their home cage and allowed to acclimate to the behavioral suite (also kept on a 12hr light:dark cycle) for 48 hours. Locomotor activity was measured with infrared photobeam detectors (Columbus Instruments) over a period of 48 hours. The two recorded 24 hr periods were averaged together for each individual animal. Locomotor activity counts in the homecage using infrared photobeams were acquired and stored using Columbus Instruments and Opto-M4 software. ***Locomotor activity in novel open field****:* Mice were handled daily 3-5 days prior to start of experiment. On each experimental day, mice were moved to behavioral suite and habituated for 30-60 min before measurements. Open-field locomotion was measured in small (20 h x 17 w x 28 l cm) polycarbonate chambers for 1hr using beak break detectors or in a large (40 x 40 x 40 cm) acrylic/PVC chambers for 30 min using video monitoring. ***Locomotor activity empty and water filled chambers***: Animals were placed in either a 20 cm diameter white chamber with nothing else added or in the same chamber that had been filled with 30°C water and were video monitored for 15 minutes. Following the swim session, animals were toweled dry to avoid hypothermia. This test was repeated during two consecutive days, once with water-filled and another day with air, counterbalanced across gentotypes. All tests were video recorded with an overhead camera and images were analyzed using Noldus Ethovision software to calculate distance travelled and speed. The mobile and immobile states were defined based on the mouse speed: > 2 cm/s for mobile, < 0.75cm/s for immobile for over 15 sample frames (3 seconds). ***Novel object exploration*****:** Following 3-5 days of handling and behavioral suite acclimation, mice were placed in open field chamber for 30 min and then returned to their homecage for 5 min, during which time a novel object was placed in the center of open field chamber. Animals were placed back into the chamber and allowed to interact with the novel object for 15 min. A 1L lab container bottle cap or a coverslip box were used as novel objects counterbalanced across mice. In a pilot experiment using Jax C57Bl6/J mice demonstrated that mice readily investigated these two objects and preferred them equally. Tests were video recorded from top view camera and videos were analyzed using Noldus Ethovision software to calculate distance travelled around object zone and speed. ***Accelerating Rotarod*****:** On a first habituation session, mice were placed on rotarod (MedAssociates) at a constant speed of 4 rpm for 1 min. During 6 daily consecutive sessions, mice were placed on accelerating rotarod (4 to 40 rpm) for a maximum of 300 seconds or until they fall. Trials were terminated if the mouse rotated with the rod >2 rotations. 6 trials were run on day one, followed by 4 trials per day, for five additional days. Each trial was separated by approximately 20 min.

Intra-striatal microinfusion of picrotoxin: Mice were "progressively" handled for five days prior to experimentation. See time course below:

Day1	Day 2	Day 3	Day 4	Day 5
Pick up	Scruff, touch head	Remove stilette	Remove stilette	Insert injector

Mice were tethered to an infusion line in which the appropriate injector was secured into a place using a cap that screwed onto the pedestal. The tether was connected to a swivel so that the animal could move freely around the arena. ACSF or picrotoxin was run through the infusion line and prior to tethering the animal sat for >10 minutes to ensure no excess liquid was coming out of the injector tip. In between each animal, the infusion system was advanced to guarantee there were no bubbles or clogs in the line. Once tethered, animals were placed in the open field chamber for a 45-minute baseline period. Following this period ACSF or picrotoxin (100 µM or 12.5 ng/100 nL, 125 nL per side for DS, 150 nL/side for NAc, 100 nL/min bilateral) (counterbalanced across two days) was infused into the striatum and locomotor activity was assessed for 25 min post-infusion**.** Animals had a day off in between infusion sessions. Picrotoxin infusion into the DS caused tic-like behavior in 20% of iMSN-Drd2KO mice in addition to enhancing horizontal locomotor activity. This may have introduced additional variation into the locomotor activity measures for DS microinfusion experiments. Tic-like behavior was not observed in the NAc microinfusion experiments. It should also be noted that rotation behavior was observed in some animals which could indicate either an imbalance in the volume of drug infused per side or be a reaction to the tether/swivel mechanism.

Gi-DREADD locomotor rescue: For systemic administration, Gi-DREADD or mCherry infected iMSN-Drd2KO mice received CNO (1 mg/kg, i.p., dissolved in saline) in their homecage 30 min prior to being placed in novel open chamber for 30 min. For intracranial administration, Gi-DREADD or mCherry infected iMSN-Drd2KO mice

received CNO (10 µM, 300 nL, 100 nL/min bilateral, dissolved in ACSF) prior to being placed in the open field chamber. The virus was allowed to diffuse for an additional 3 minutes and then animals were placed (untethered) in the open field for 70 minutes. Note, that for these intracranial experiments, the same "progressive" handling procedure was used that was used for the picrotoxin microinfusion experiments.

Electrophysiology: Sagittal slices (240 µm) containing the dorsomedial striatum and NAc core were prepared from *Drd2*$^{loxP/loxP}$ and iMSN-Drd2KO mice (8-16 wk old) that were crossed into the *Drd1-tdTomato* strain. Recordings from control and KO mice were interleaved across days and animals were age- and sex-matched. Slices were cut in ice cold cutting solution (in mM): 225 sucrose, 13.9 NaCl, 26.2 $NaHCO_3$, 1 NaH_2PO_4, 1.25 glucose, 2.5 KCl, 0.1 $CaCl_2$, 4.9 $MgCl_2$, and 3 kynurenic acid. Slices were maintained in oxygenated ACSF containing (in mM): 124 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 26.2 $NaHCO_3$, 1 NaH_2PO_4, and 20 glucose (~310-315 mOsm) at RT. Slices were placed in a recording chamber and continuously perfused with oxygenated ACSF at approximately 1.5-2 ml/min, maintained at 30-32˚C by an inline solution heater (TC-324B, Warner Instruments). Recordings were made in the dorsal and ventral striatum from td-Tomato positive and negative neurons (putative dMSNs and iMSNs respectively). Cells were excluded if they had a membrane resistance of <100 MΩ or >500 MΩ or were spontaneously active to reduce the probability of recording from either interneurons or dying cells. Current clamp recordings were performed using electrodes (2.5-4 MΩ) filled with 120 $KMeSO_4$, 20 KCl, 10 HEPES, 0.2 K-EGTA, 2 $MgCl_2$, 4 Na-ATP, and 0.4 Na-GTP (pH = 7.25, ~290 mOsm). During the current injection protocol, cells were maintained at -80 mV. Cells were injected with current for 800 msec ranging from -160 to 260 pA. These experiments were done in the presence of NBQX (5 µM), Gabazine (5 µM), CPP (5 µM). Whole cell voltage clamp recordings measuring mIPSCs used electrodes (2-4 MΩ) filled with internal solution containing in mM: 60 $CsMeSO_3$, 60 CsCl, 10 HEPES, 0.2 EGTA, 8 NaCl, 2$MgCl_2$, 2 Mg-ATP, 0.3 Na-GTP, and 10 phosphocreatine (pH = 7.24, ~300 mOsm). This internal shifted the E_{Cl-} to -19.4 mV, which allowed for detection of GABA-A receptor mediated inward currents. Experiments were done in the presence of NBQX (5 µM), CPP (5 µM), CGP 55845 (2 µM), and tetrodotoxin (TTX) (1 µM). Gabazine at either 5 or 10 µM (for tonic current) completely blocked mIPSCs and the slow tonic currents. Whole cell voltage clamp recordings measuring mEPSCs used electrodes (2-4 MΩ) filled with internal solution containing in mM: 120 $CsMeSO_3$, 10 HEPES, 0.2 EGTA, 8 NaCl, 2$MgCl_2$, 2 Mg-ATP, 0.3 Na-GTP, and 10 phosphocreatine (pH = 7.24, ~290 mOsm). These experiments were done in the presence of Gabazine (5 µM), CPP (5 µM), CGP 55845 (2 µM) and tetrodotoxin (TTX) (1 µM). Access resistance was monitored throughout the experiment. All groups had comparable average access resistance. Data was acquired at 5 kHz and filtered at 1 kHz using Multiclamp 700B (Molecular Devices). Data was analyzed using pClamp (Clampfit, v. 10.3) and MiniAnalysis (Synaptosoft, Inc.). For detection of mIPSCs, thresholds were 9-12 pA depending on the noise and area ranged from 30 to 50. Thresholds were similar across cell types and genotypes (data not shown). For detection of mIPSCs, thresholds were 5-7 pA depending on the noise and area ranged from 10 to 20. Thresholds were similar across cell types and genotypes (data not shown).

Fast scanning cyclic voltammetry: Brain slices and solutions were prepared as described in the electrophysiology section and recordings performed as previously described (Adrover et al., 2014). The carbon-fiber electrode was held at −0.4 V and a voltage ramp to and from 1.2 V (400V/s) was delivered every 100 ms (10 Hz). Before recording, electrodes were conditioned by running the ramp at 60 Hz for 15 min and at 10 Hz for another 15 min and calibrated. Electrodes were calibrated using 1 µM DA. DA transients were evoked by electrical stimulation delivered through a glass microelectrode filled with ACSF. Either a single monophasic pulse (0.2msec, 300 µA) or train of pulses (20 pulses, 20Hz) was delivered to the slice in the absence or presence of the D2R antagonist sulpiride (1 µM). Data were collected with a retrofitted CB-7B/EC headstage (with 5 MΩ resistor) using a Multiclamp 700B amplifier, digitized at 100 kHz and filtered with low-pass filter at 10 kHz using pClamp10 software (all from Molecular Devices). Voltammetric analysis was done using custom written procedures in Igor Pro software.

***In* vivo Electrophysiology:** iMSN-Drd2KO mice or control mice (*Adora2A-Cre*$^{+/-}$ mice or *Drd2*$^{loxP/loxP}$) were anesthetized with chloral hydrate (380 mg/kg) during surgery. In a subset of animals, ChR2 or KOR-DREADD was infused into the striatum (A/P: +0.8, Lat: +1.5, D/V: -2.7 mm from top of skull) of *Adora2A-Cre*$^{+/-}$ or iMSN-Drd2KO mice. One array of 32 Teflon-coated tungsten microwires (35 µm diameter) was implanted unilaterally in striatum (A/P: +0.8, Lat: +1.5, D/V: -2.6 mm from top of skull) or the globus pallidus (A/P:-0.5, Lat: +2.0, D/V:-3.5 from top of skull) and a stainless steel ground wire was implanted under the skull. For recordings, animals were transported to the recording room and placed in a 25 cm plastic chamber or a novel mouse cage, and allowed to move freely. All recording experiments except experiments conducted during KOR-DREADD activation were 1hr

in duration and there were 1-4 recordings per mouse. Electrodes that detected a unit on one recording session were not included in future recording sessions to avoid over-sampling from the same neurons. For KOR-DREADD experiments, animals were recorded for 1hr, injected with either saline or salvinorin B (17mg/kg dissolved in DMSO), and recorded for an additional 2 hrs during which electrical signals were sampled, digitized, time-stamped, and stored for offline analysis using a Plexon recording system (Plexon, inc.). SalB and DMSO injections were performed on separate days separated by 1 week in a counter-balanced design. Single units were discriminated using principal component analysis (Offline Sorter; Plexon). Spike channels were acquired at 40kHz with 16 bit resolution, and the signal was band pass filtered at 150 Hz-8 kHz before spike sorting. Medium spiny neurons and interneurons were identified based on waveform shape and average firing rate, similar to what has previously been described (Berke et al., 2004). High-quality single unit isolation was achieved, as assessed by measures of spike-sorting quality (Wheeler 1998) Davies–Bouldin index, J3 statistic.

Immunohistochemistry: Mice were intracardially perfused with PBS containing 10U/mL Heparin until the liver cleared (approximately 5 minutes) followed by 40 ml fixative solution (4% paraformaldehyde; 4% sucrose in 0.1M PB) at 3 ml/min. Brains were incubated in 30% sucrose (in 0.1 M PB) for 24 h at 4 °C and sliced with a crysostat. 40µm floating sections were washed in PBS and then blocked for 1hr in 5% normal goat serum or donkey serum, 0.3% Triton-X in PBS at RT. Sections were then incubated in primary antibody for 12-18hrs at RT. Slices were incubated in goat or donkey Alexa Fluor secondary antibodies (1:500, Invitrogen) for 2 hr at RT. Slices were washed 3 x 10 min in PBS, then 2 x 10 min in 0.1M PB. Images (512x512 or 1024x1024) were acquired using a confocal microscope (Zeiss LSM 510 META, Thornwood, NY). Two-three confocal images were acquired per region (throughout NAc and dorsal striatum, and throughout VTA/SNc when stated) per mouse, with 3-4 mice of each genotype and condition. Analysis was performed using ImageJ software on acquired images.

Antibodies used in this study

Antibody	**Concentration**	**Host**	**Marker for?**	**Source**	**Catalog number**
α-met-enkaphalin	1:100	Rabbit	iMSNs	Immunostar	#20065
α-tyrosine hydroxylase	1:1000	Mouse	Dopamine neurons	Sigma-Aldrich	T2928
α-choline acetyltransferase	1:300	Goat	Cholinergic interneruons	Millipore	#AB144P

Statistics: Statistical analysis was performed in Prism (GraphPad) and Excel. Two-tailed unpaired t-tests were used unless stated. Otherwise, two-tailed paired t-test, one-Way ANOVAs or two-Way Repeated Measures (RM) ANOVAs or one-tailed t-tests were used when appropriate and stated. One-tailed t-tests were used sparingly and only when we had an *a priori* hypothesis of a unidirectional effect. Two-way ANOVAs were followed up with Sidak-corrected t-test comparisons. All data are presented as mean ± SEM. Results were considered significant at an alpha of 0.05. Unless otherwise specified n = number of cells for electrophysiology data and number of animals for behavioral data.

Supplemental References

1. Adrover, M.F., Shin, J.H., and Alvarez, V.A. (2014). Glutamate and dopamine transmission from midbrain dopamine neurons share similar release properties but are differentially affected by cocaine. J Neurosci *34*, 3183-3192.
2. Bello, E.P., Mateo, Y., Gelman, D.M., Noain, D., Shin, J.H., Low, M.J., Alvarez, V.A., Lovinger, D.M., and Rubinstein, M. (2011). Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. Nature neuroscience *14*, 1033-1038.
3. Berke, J.D., Okatan, M., Skurski, J., and Eichenbaum, H.B. (2004). Oscillatory entrainment of striatal neurons in freely moving rats. Neuron *43*, 883-896.
4. Gerfen, C.R., Paletzki, R., and Heintz, N. (2013). GENSAT BAC cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. Neuron *80*, 1368-1383.
5. 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. The Journal of neuroscience : the official journal of the Society for Neuroscience *28*, 2681-2685.