Supplementary Materials and Methods

Ca^{2+} channel current recording

Dopamine neurons were first identified using loose-patch firing recordings in normal extracellular solution containing (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 11 glucose, and 25 $NaHCO_3$, saturated with 95% O_2 and 5% CO_2 (pH 7.4, ~295 mOsm/kg). Slices were then perfused with a solution containing $BaCl_2$ (2.4 mM; substituting $CaCl_2$ to remove Ca^{2+}-dependent inactivation of LTCCs and to block various K^+ channels), TTX (250 nM), and ZD7288 (I_h antagonist; 10 μM). Whole-cell recordings were made with an internal solution containing containing Cs-methanesulfonate (115 mM; in place of K-methylsulfate to block various K^+ channels). To evoke Ca^{2+} channel currents, 1–1.5 s depolarizing steps of 10–15 mV were applied every 30 s from a holding potential of –62 mV, where low-threshold T-type Ca^{2+} channel currents were largely inactivated (Supplementary Figure S1). Leak currents were continuously monitored using three 5 mV hyperpolarizing pulses from a holding potential of –87 mV and subtracted offline. Series resistance was compensated by ~70%. Total inward currents thus recorded were completely blocked by Cd^{2+} (500 μM), a nonselective Ca^{2+} channel antagonist (n = 3).

Flash Photolysis

Cells were dialyzed with caged IP_3 (20–400 μM) through the whole-cell pipette for 15–20 min after break-in. A UV flash (~1 ms) was applied with a xenon arc lamp to photolyze caged IP_3. The UV flash was focused through a 60× objective onto a 350 μm area surrounding the recorded neuron. UV flash intensity was varied to alter the amount of IP_3 released into the cytosol.

Intra-VTA microinjections

Rats (8–9 weeks old) were anesthetized with a mixture of ketamine and xylazine (90 mg/kg and 10 mg/kg, i.p.) and implanted with bilateral chronic guide cannulas (22 gauge; Plastics One), with dummy cannulas (32 gauge) inside, aimed at 1 mm above the VTA (anteroposterior, –5.3; mediolateral, +2.2; dorsoventral, –7.5; 10° angle)[1]. The guide cannulas were fixed to the skull with stainless steal screws and dental cement. After the surgery, rats remained singly housed for 7 days before being subjected to CPP experiments.

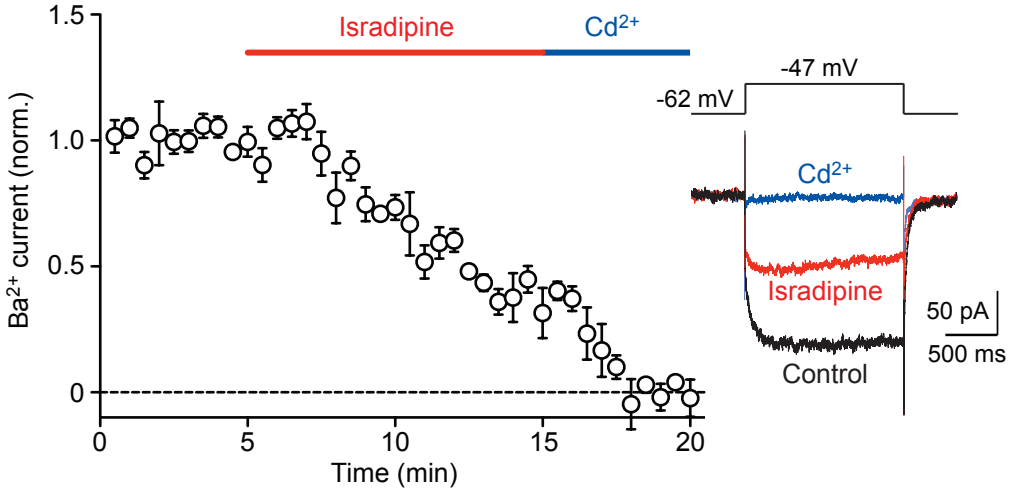
Intra-VTA microinjections were made via injection cannulas (28 gauge; Plastics One) that extended 1 mm beyond the tip of the guide cannulas. Injection cannulas were connected to 1 μl Hamilton syringes mounted on a microdrive pump (Harvard apparatus). Rats received bilateral infusions (0.3 μl/side, 0.15 μl/min) of different pharmacological agents in certain CPP experiments. The injection cannulas were left in place for 60 s after infusion.

At the end of CPP experiments, rats were anesthetized with a mixture of ketamine and xylazine (90 mg/kg and 10 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde. Brains were then carefully removed and stored in 4% paraformaldehyde. Coronal sections (100 μm) were cut using a vibratome and stained with cresyl violet for histological verification of injections sites (Supplementary Figure S13). Data from rats with injection sites outside the VTA were excluded from the analysis.

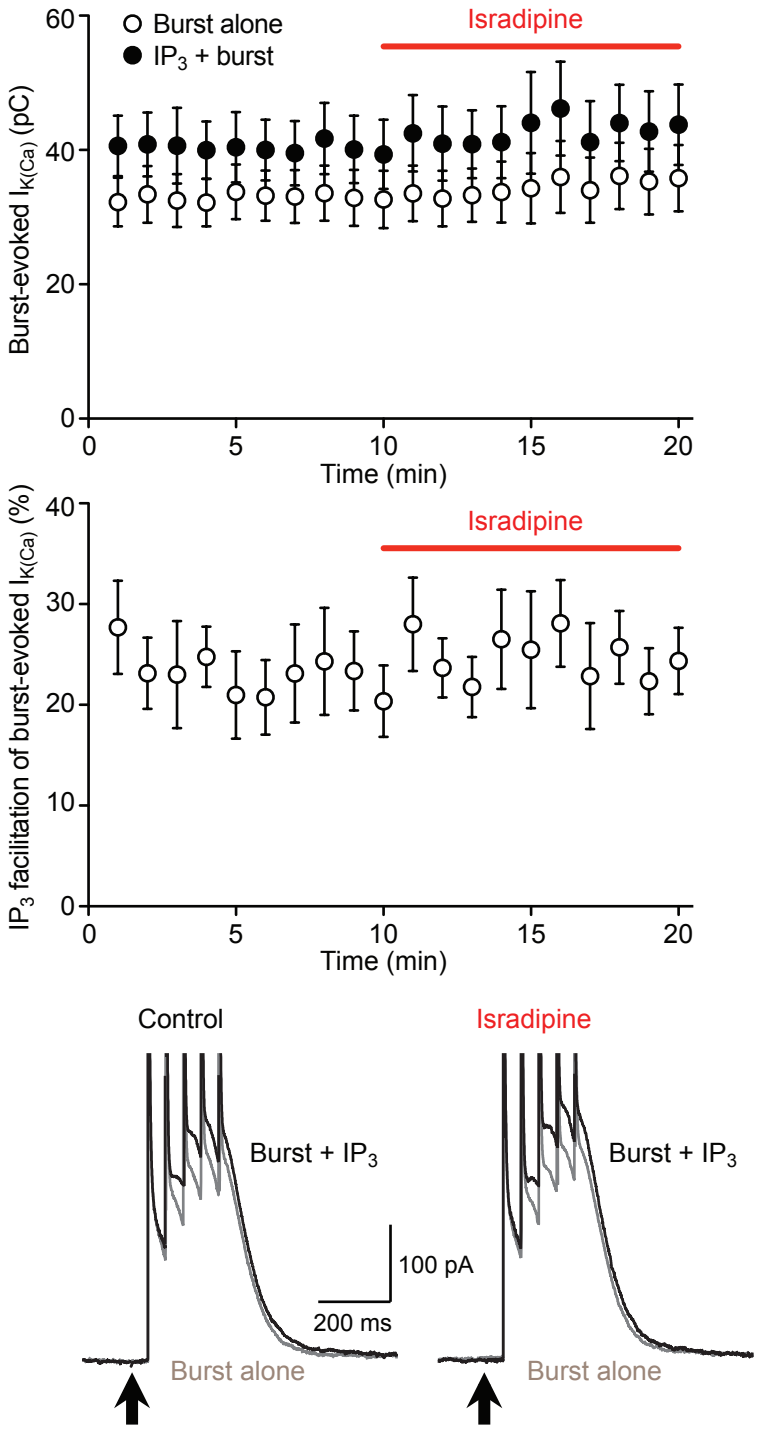
Drugs

Isradipine, DNQX, picrotoxin, CGP55845, eticlopride, and S(-)-Bay K 8644 were obtained from Tocris Biosciences. Compound 8 and caged IP_3 were generous gifts from Dr. D. James Surmeier (Northwestern University) and Dr. Kamran Khodakhah (Albert Einstein College of Medicine). All other chemicals were from Sigma-RBI.

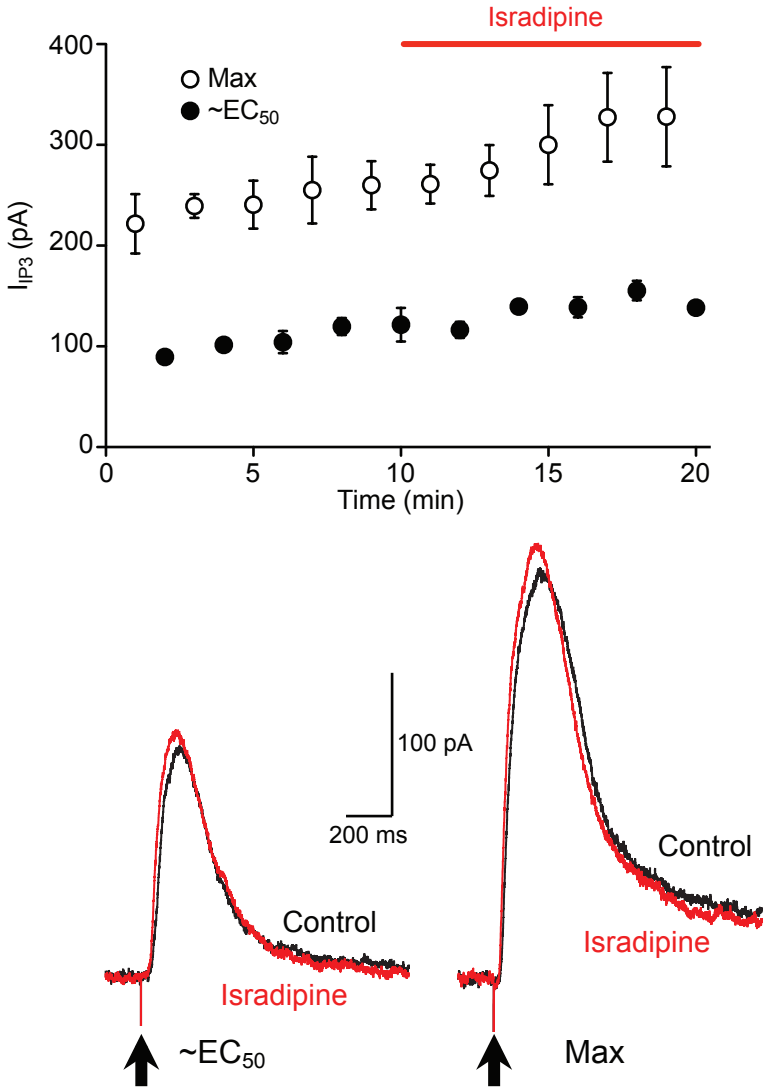
1. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th edn. Academic Press: San Diego, 1998.



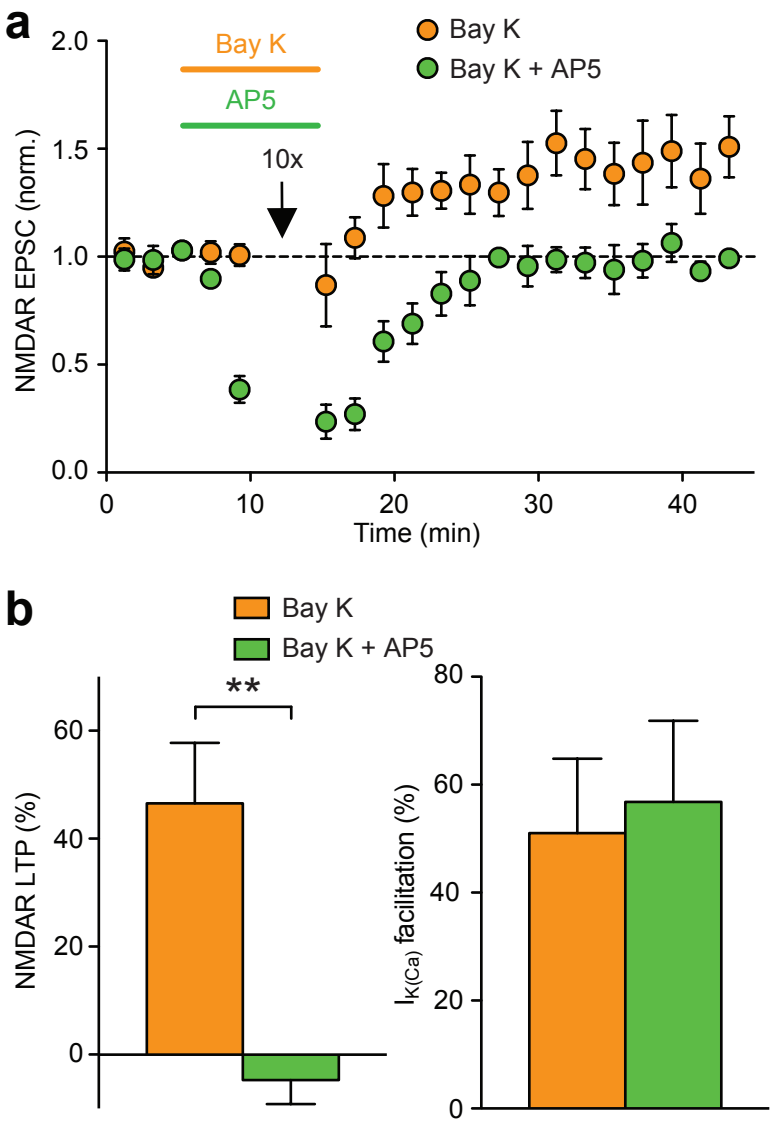
Supplementary Figure S1. Isradipine inhibits Ca^{2+} channel currents in VTA dopamine neurons. Summary time graph depicts the effects of isradipine (2 μM) and Cd^{2+} (500 μM) on Ca^{2+} channel currents recorded using Ba^{2+} as charge carrier (n = 4). Example averaged traces of Ba^{2+} currents are shown on the right. Ba^{2+} current amplitude was measured from a 100 ms window close to the end of the voltage step.



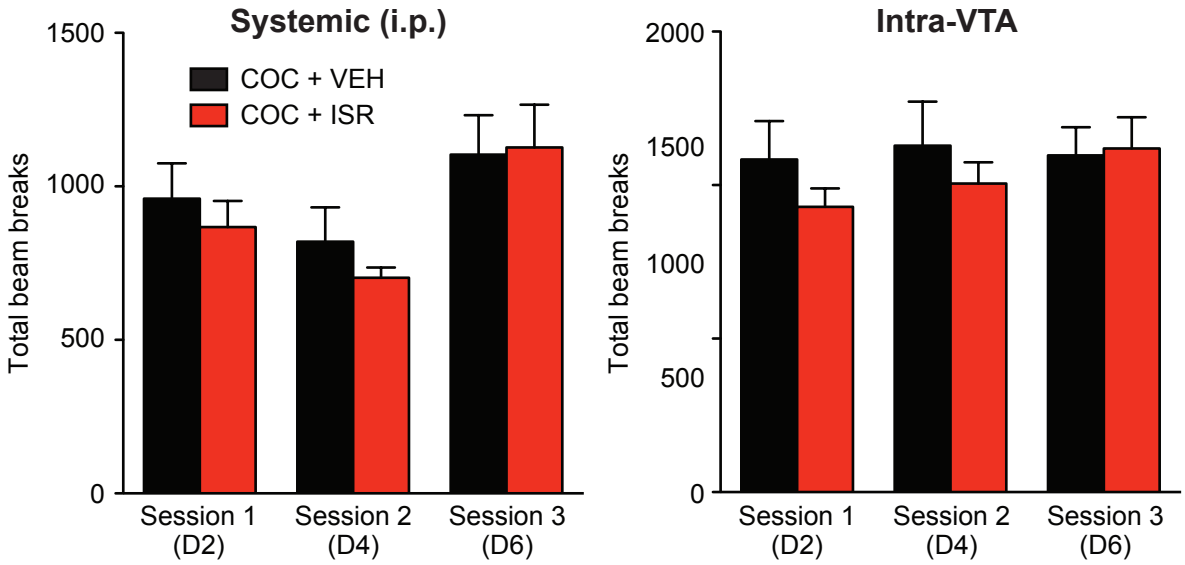
Supplementary Figure S2. Isradipine has no effect on burst-evoked $I_{K(Ca)}$ and its facilitation produced by IP_3. Summary time graphs showing the effect of isradipine (2 μM) on burst-evoked $I_{K(Ca)}$ (without or with preceding IP_3 application) and on the degree of IP_3-induced facilitation of $I_{K(Ca)}$ (n = 8). Example traces of burst-evoked $I_{K(Ca)}$ in control and in isradipine are shown at the bottom. UV photolysis of caged IP_3 (25 μM in the pipette) was made at the time indicated by the arrow. UV pulse intensity was adjusted to evoke minimal outward current by itself (<15 pA).



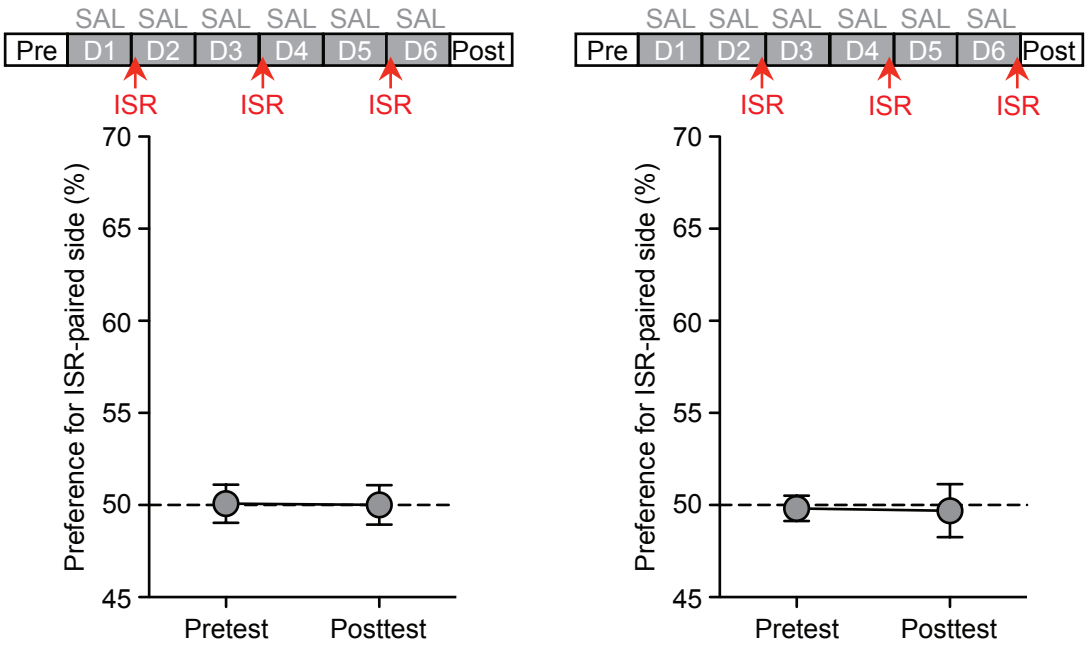
Supplementary Figure S3. Isradipine has no effect on IP_3-evoked outward currents (I_{IP3}). Summary time graph showing the effect of isradipine (2 μM) on I_{IP3} (n = 3). Example traces depicting the effect of isradipine on I_{IP3} are shown at the bottom. UV photolysis of caged IP_3 (400 μM in the pipette) was made at the time indicated by the arrow. UV pulse intensity was adjusted to evoke maximal outward current and ~50% of the maximal amplitude (~EC_{50}) in each cell.



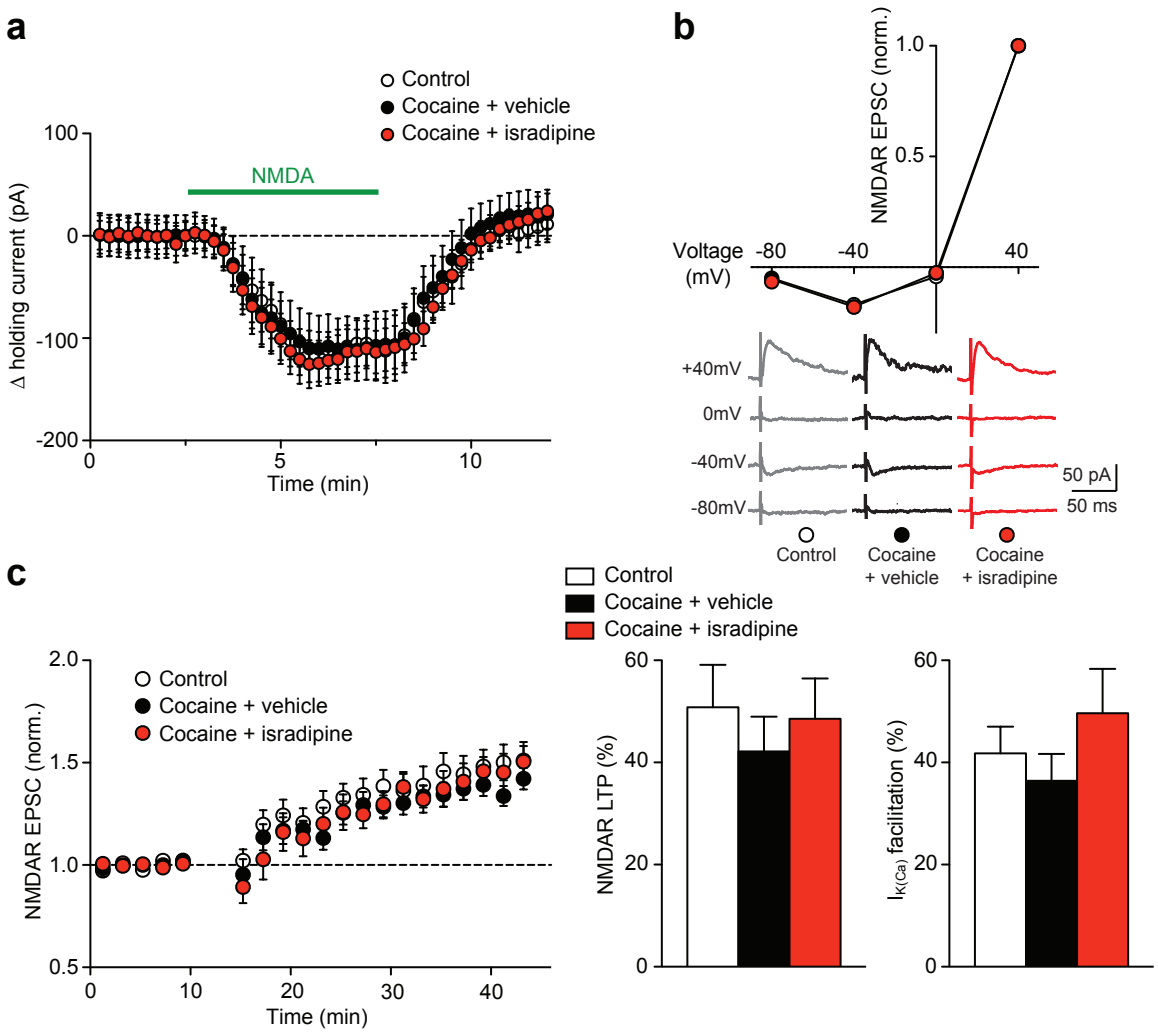
Supplementary Figure S4. LTCC activation with Bay K 8644 has no facilitatory effect on NMDAR LTP induction. (a) Summary time graph of LTP experiments in which LTP induction protocol was delivered in Bay K 8644 (1 μM; n = 4) or in Bay K 8644 and AP5 (5 μM; produced 86 ± 8% inhibition of EPSCs, n = 4). (b) AP5 blocked LTP induction in Bay K 8644 without affecting synaptic facilitation of $I_{K(Ca)}$. Data are from the same experiments shown in (a) (LTP: $t_6 = 4.25$, $p < 0.01$; $I_{K(Ca)}$ facilitation: $t_6 = 0.28$, $p = 0.79$; unpaired t test).



Supplementary Figure S5. Summary graphs depicting the overall activity level, i.e., total number of beam breaks in the CPP box compartment, during the three cocaine conditioning sessions for the experiments show in Figure 1a, where isradipine was injected before each conditioning session (systemic: $F_{2,60} = 0.33$, $p = 0.72$, n = 16 rats/group; intra-VTA: $F_{2,24} = 0.71$, $p = 0.50$, n = 6–8 rats/group; mixed two-way ANOVA).

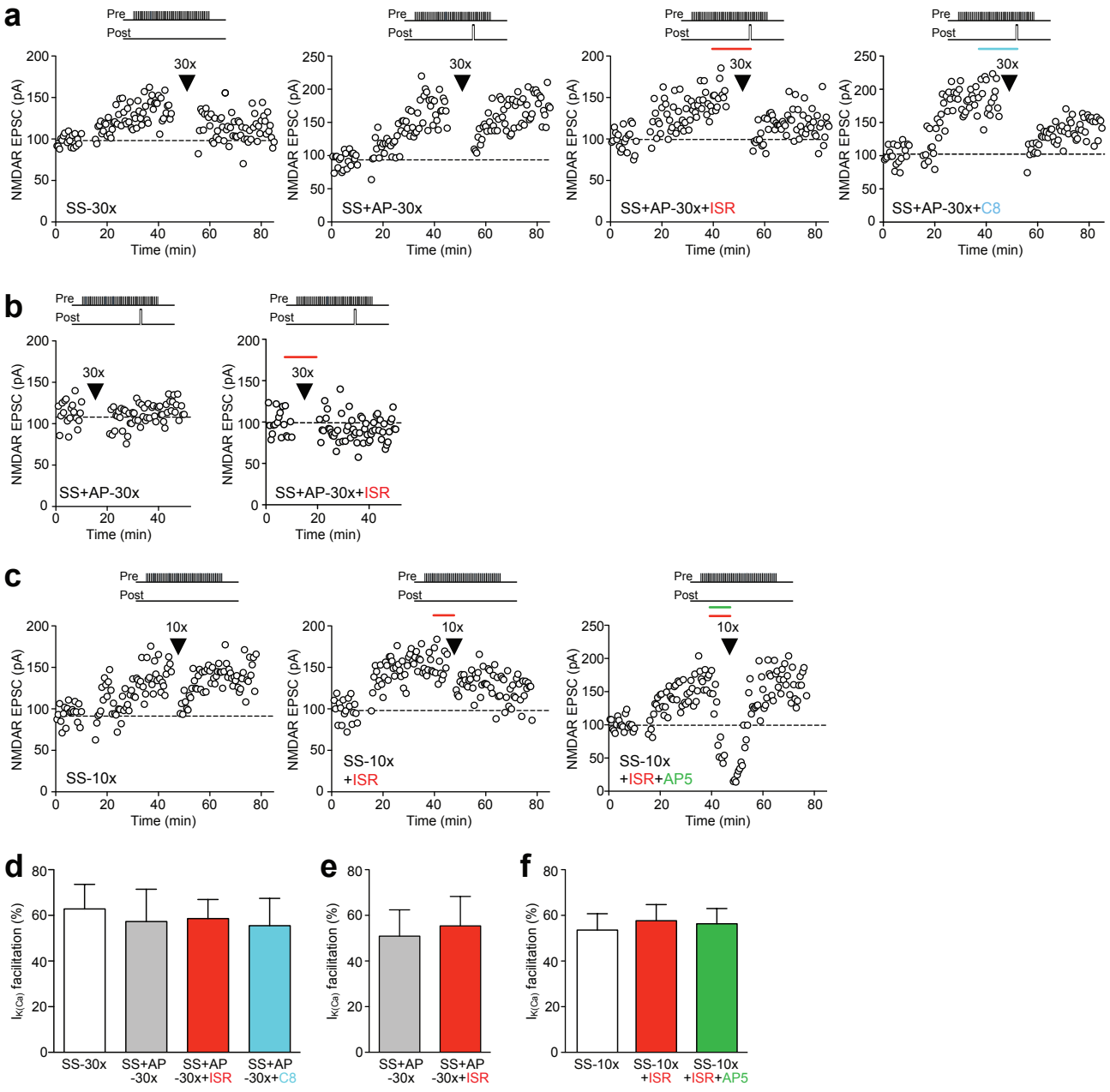


Supplementary Figure S6. Intra-VTA isradipine injection had no effect on side preference by itself. Both sides were paired with i.p. injection of saline (SAL), while only one side was paired with intra-VTA isradipine (ISR) injection made before (left) or after (right) being placed in one compartment of the CPP box (left: $t_7 = 0.09$, $p = 0.93$, $n = 8$ rats; right: $t_7 = 0.12$, $p = 0.91$, $n = 8$ rats; paired t test).

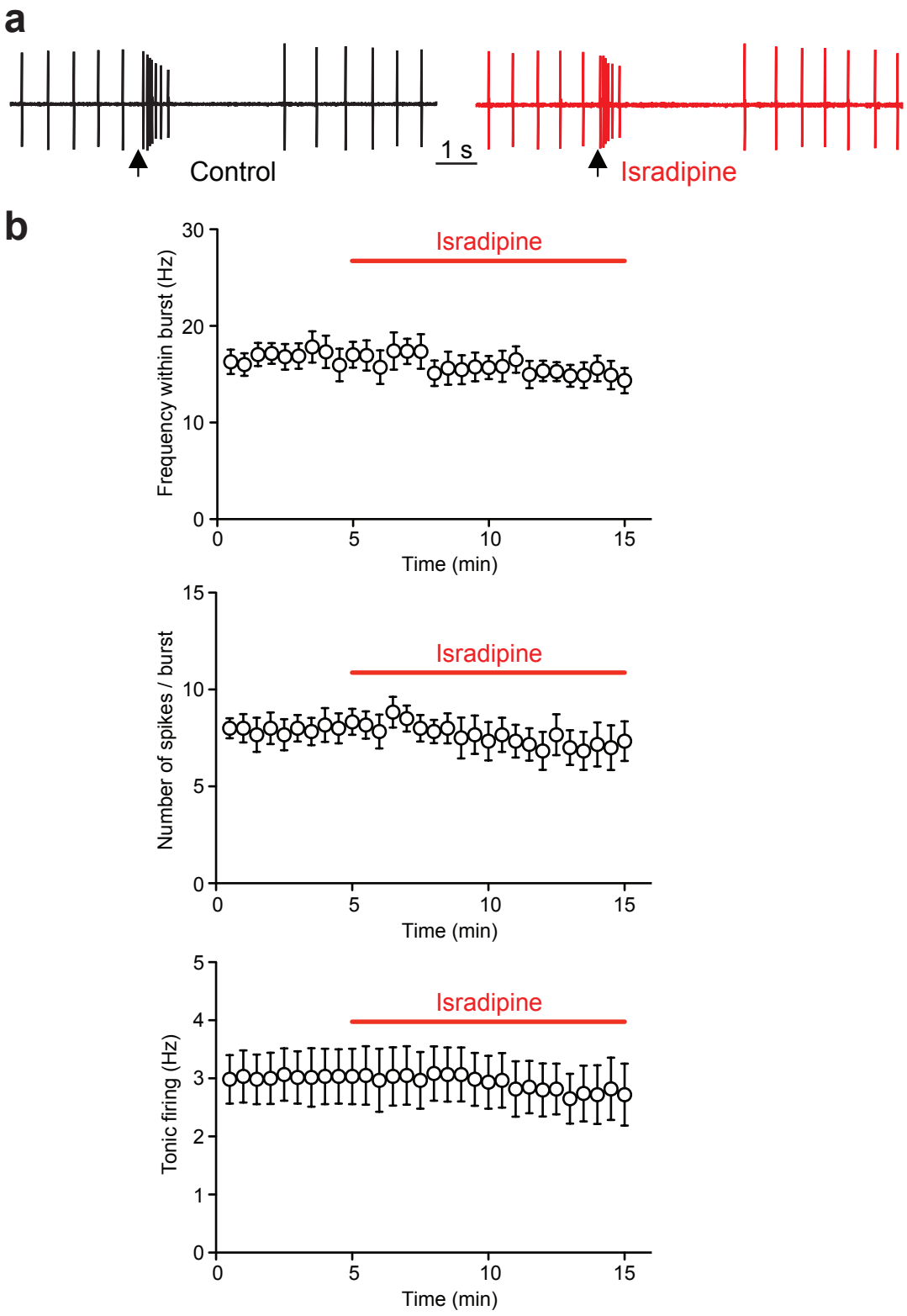


Supplementary Figure S7. Cocaine conditioning does not result in global changes in NMDAR-mediated transmission. (a) Summary time graph depicting the inward current produced by bath application of NMDA (10 μM) in control rats and in rats that have undergone cocaine conditioning with systemic (i.p.) injection of isradipine or vehicle. (b) I-V relationship of NMDAR EPSCs (amplitude normalized to the value at +40 mV) recorded in normal extracellular Mg^{2+} (1.2 mM) with Cs-based intracellular solution. Representative EPSC traces are shown at the bottom. (c) Summary time graph of NMDA LTP experiments and bar graphs showing LTP magnitude and degree of synaptic facilitation of $I_{K(Ca)}$. LTP data in the control group are from cells shown in Figures 1c and d. Note that enhanced LTP associated with increased facilitation of $I_{K(Ca)}$ observed after amphetamine treatment in our previous study (Ahn et al., 2010) was not seen in the present study after cocaine conditioning. This is most likely due to the difference in synaptic stimulation intensity. In the previous study, synaptic stimulation intensity was adjusted to evoke NMDAR EPSCs of ~40 pA (as opposed to ~100 pA in the present study), which resulted in little synaptic facilitation of $I_{K(Ca)}$ and thus little LTP in control rats.

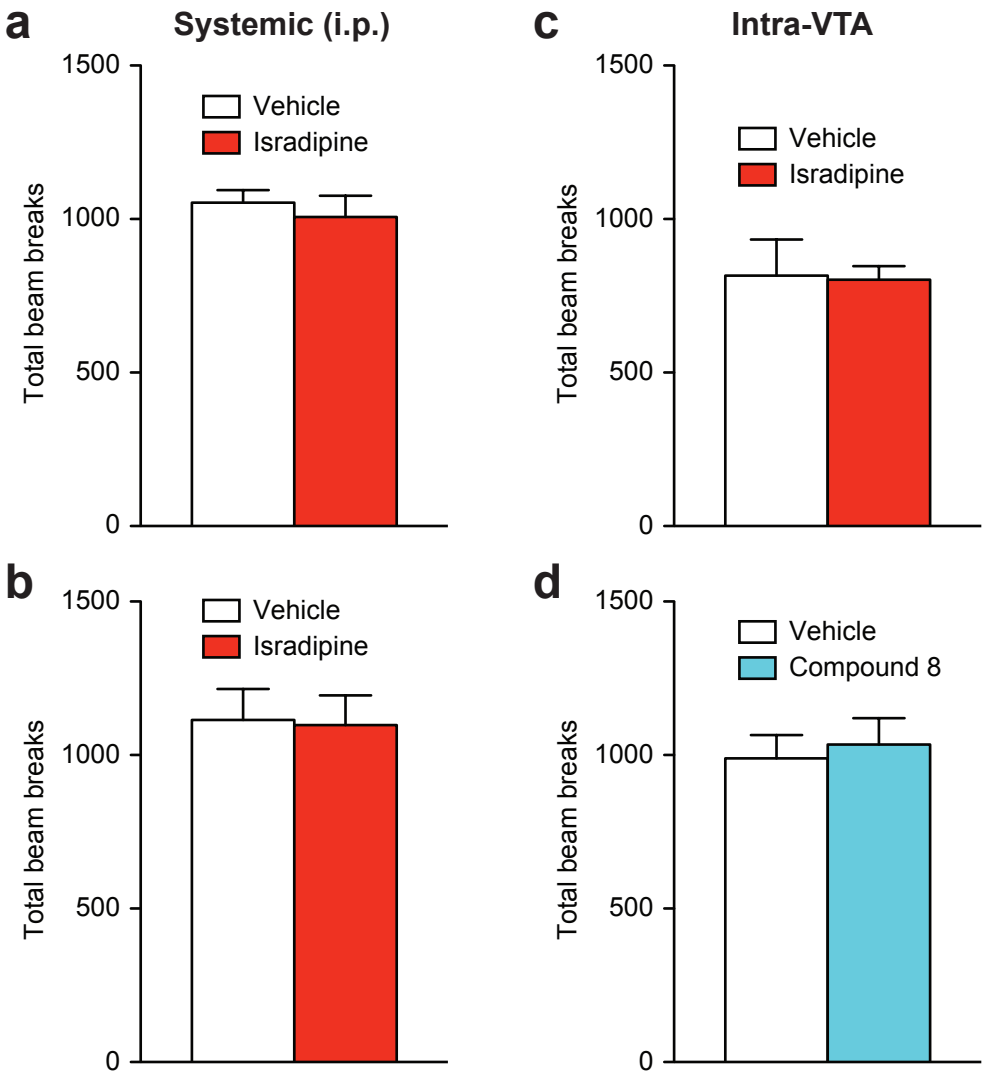
Ahn KC, Bernier BE, Harnett MT, Morikawa H. IP_3 receptor sensitization during in vivo amphetamine experience enhances NMDA receptor plasticity in dopamine neurons of the ventral tegmental area. J Neurosci 2010; 30: 6689-6699.



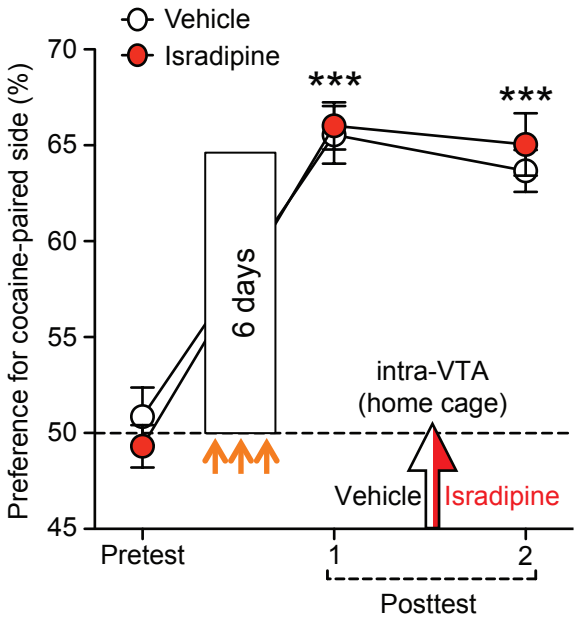
Supplementary Figure S8. Isradipine and compound 8 promotes reversal of previously induced NMDAR LTP. (a-c) Time course of example experiments shown in Figures 3a-c. LTP reversal protocol depicted at the top of each graph was repeated 30 times (a and b) or 10 times (b). Isradipine, compound 8, and AP5 were perfused at the time indicated by horizontal lines. (d-f) Summary bar graphs demonstrating that the degree of synaptic facilitation of $I_{K(Ca)}$ measured after 10 min baseline recording was not different among groups shown in Figures 3a-c.



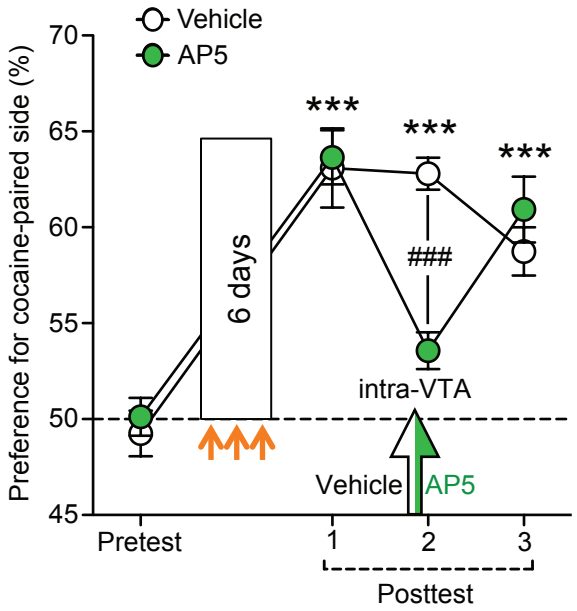
Supplementary Figure S9. Example traces (a; aspartate iontophoresis at arrows) and summary time graphs (b) illustrating that isradipine had no effect on the frequency/number of spikes within the burst (top/middle panels) or tonic firing (bottom panel) after cocaine conditioning (n = 6 cells). Recordings were made in VTA slices prepared from rats that had undergone 3-day cocaine conditioning with systemic injection of vehicle before each conditioning session (Figure 2a, left panel).



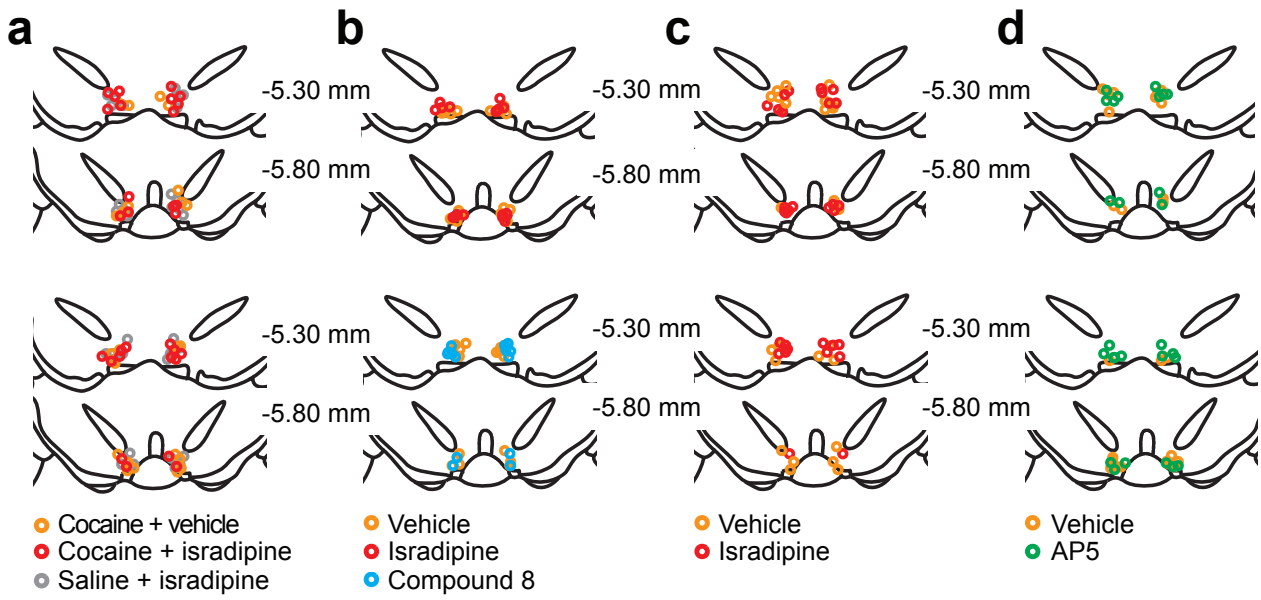
Supplementary Figure S10. Summary graphs depicting the overall activity level, i.e., total number of beam breaks in the CPP box, during the second posttest for the experiments show in Figure 4a-d, where isradipine (a-c) or compound 8 (d) was injected before the second posttest (a: cocaine CPP with i.p. isradipine, b: ethanol CPP with i.p. isradipine, c: cocaine CPP with intra-VTA isradipine, d: cocaine CPP with intra-VTA compound 8).



Supplementary Figure S11. Intra-VTA injection of isradipine in home cage does not promote extinction of cocaine CPP. Summary graph of CPP experiments in which intra-VTA injection of vehicle/isradipine was made in the home cage ($F_{2,20}$ = 0.57, p = 0.58, n = 6 rats/group; mixed two-way ANOVA). ***p < 0.001 vs. pretest (Bonferroni post hoc test).



Supplementary Figure S12. Intra-VTA AP5 blocks expression of cocaine CPP. Summary graph of CPP experiments in which intra-VTA injection of vehicle/AP5 was made before the second posttest ($F_{3,36}$ = 8.77, p < 0.001, n = 7 rats/group; mixed two-way ANOVA). ***p < 0.001 vs. pre-test; ###p < 0.001 between groups (Bonferroni post hoc test).



Supplementary Figure S13. Approximate locations (mm from bregma) of cannula tips for intra-VTA microinjection experiments. (a) Top: experiments in Figure 2a; bottom: experiments in Figure 2b. (b) Top: experiments in Figure 4c; bottom: experiments in Figure 4d. (c) Top: experiments in Figure 4e; bottom: experiments in Supplementary Figure S11. (d) Top: experiments in Figure 4f; bottom: experiments in Supplementary Figure S12.