ONLINE METHODS

Generation and breeding of conditional *Drd2* mutant mice. We generated mutant mice carrying two targeted loxP sites flanking *Drd2* exon 2 (*Drd2*^{tm1Mrub}, also known as *Drd2*$^{loxP/loxP}$) as described in **Supplementary Figure 1**. Mice lacking DA D_2 autoreceptors were obtained by crossing *Drd2*$^{loxP/loxP}$ with *Dat*$^{+/IRES-cre}$ mice[25] that express Cre under the transcriptional control of the endogenous *Dat* locus. We backcrossed both parental lines with C57BL/6J mice for ten generations to homogenize their genetic background (n = 10). We mated *Drd2*$^{loxP/loxP}$; *Dat*$^{+/IRES-cre}$ mice with *Drd2*$^{loxP/loxP}$ mice to establish a colony that produced equal amounts of *Drd2*$^{loxP/loxP}$; *Dat*$^{+/IRES-cre}$ mice and *Drd2*$^{loxP/loxP}$ control littermates. We genotyped mice by PCR using primers to detect the presence 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 *Cre* recombinase gene sequence (5′-AAA ATT TGC CTG CAT TAC CG-3′ and 5′-GCA TAA CCA GTG AAA CAG CAT TGC TG-3′). We used adult male mice except where noted.

Animal husbandry. We housed same-sex animals of mixed genotypes in groups of up to six animals per cage in an animal room at 22 °C under a 12-h light/dark cycle (lights on at 7:00 a.m.), with *ad libitum* access to food and water (except when noted). We conducted all testing during the light period and between 11:00 a.m. and 6:00 p.m. Animal protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, United States Public Health Services and Animal Care and Use Committee of the School of Sciences, University of Buenos Aires. Electrophysiology and electrochemistry experiments were performed in accordance with guidelines from the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use Committee.

D_2 receptor autoradiography. D_2 receptor autoradiography was performed as described previously[42], with modifications. We incubated frozen brain and pituitary 16–20-µm coronal sections in binding buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH = 7.4) containing 1 nM [^{3}H]nemonapride (NEN PerkinElmer). We determined nonspecific binding in the presence of 10 µM *S*-(−)-sulpiride. We then washed the sections, dried them and exposed them to BIOMAX MR film (Kodak) for 4 weeks.

***In situ* hybridization with digoxigenin-labeled RNA probes.** We processed 16-µm cryostat sections for *in situ* hybridization according to previously described protocols[43,44]. We prepared an *in vitro* transcribed digoxigenin-UTP–labeled antisense riboprobe for mouse *Drd2* exon 2 according to instructions of the manufacturer (Roche).

Electrophysiology. We prepared 220-µm horizontal slices from midbrain of auto-Drd2KO mice and *Drd2*$^{loxP/loxP}$ control littermates (3–5 months old). Slices were recovered for 20 min at 33 °C and placed in a submerged chamber perfused at 2 ml min^{-1} with standard artificial cerebrospinal fluid containing 124 mM NaCl, 1 mM NaH_2PO_4, 2.5 mM KCl, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 20 mM glucose, and 26.2 mM $NaHCO_3$ at 31 °C using an in-line heater (Harvard Apparatus). We performed visualized whole-cell patch-clamp recording using recording electrodes, with a resistance ~2 MΩ, that were filled with a solution containing 115 mM potassium methylsulfate, 10 mM BAPTA, 10 mM sodium phosphocreatine, 10 mM KCl, 1.5 $MgCl_2$, 10 mM HEPES, 4 mM Na-ATP, and 0.4 mM Na-GTP (pH 7.2–7.3). We used interpeduncular fossa and medial terminal nucleus as landmarks to locate midbrain DA neurons, which were further confirmed by spontaneous activities at 2–5 Hz and I_h current. Cells were voltage clamped at −55 mV and the currents were low-pass filtered at 1 kHz and digitized at 5 kHz. For extracellular stimulation, standard patch pipettes filled with artificial cerebrospinal fluid were placed ~100 µm caudal to the cell being recorded. We applied a total of 10 1-ms-long pulses at 100 Hz to evoke a postsynaptic slow IPSC in the presence of NBQX (10 µM), MK-801 (10 µM), gabazine (5 µM) and prazosin (100 nM).

Fast-scan cyclic voltammetry. FSCV was performed in mouse coronal slices containing the dorsal striatum[45]. We prepared cylindrical carbon-fiber microelectrodes (75–100 µm of exposed fiber) with T650 fibers (6-µm diameter) inserted into a glass pipette. The carbon-fiber electrode was held at −0.4 V versus Ag/AgCl and periodically a cyclic voltammogram was acquired (100-ms intervals). The applied potential was scanned to +1.2 V and back in a triangular fashion at 400 V s^{-1}. DA was evoked by single pulse or train stimulations. For single pulse stimulation, we applied a rectangular, electrical pulse (50–600 µA, 0.6 ms per phase, biphasic) every 5 min. For train stimulation, we delivered a 10-Hz train of 30 pulses (600 µA, biphasic) at 10-min intervals. Ten cyclic voltammograms were recorded before stimulation, averaged and subtracted from the cyclic voltammograms collected. Once the extracellular DA response to electrical stimulation was stable for five successive stimulations, we applied drugs to the brain slice via the superfusate. We performed each test in one slice, which served as its own precondition control and the three conditions were recorded in the same recording site from the same animal.

L-DOPA determination. We treated mice with saline or the DOPA-decarboxylase inhibitor NSD-1015 (3-hydroxybenzylhydrazine, 100 mg per kg, intraperitoneal) and killed them after 40 min. We determined L-DOPA and DA contents as described previously[46] by high-performance liquid chromatography coupled with an electrochemical detector. We quantified each striatum separately and then averaged them before performing statistical analysis.

Open field test. We evaluated horizontal locomotion and exploration in a novel open field in activity boxes (Med Associates) for 60 min for 3 consecutive days. We determined total distance, habituation, total ambulatory episodes, average velocity and periphery/center trajectories. In pharmacological experiments, mice received intraperitoneal injections with either saline (154 mM NaCl) or the corresponding drug and then placed in the open field to measure their locomotor activity. When giving haloperidol (Tocris), we recorded mouse activity 20 min after the injection. For quinpirole (Sigma) and cocaine (Sigma), we recorded mouse activity immediately after injections.

Rotarod and elevated plus maze. Performance were described previously[29]. For the rotarod test, we trained mice (n = 6–7) for 3 consecutive days in an elevated rotating rod (Med Associates) at 16 rpm. The third day, 30 min after the last training session, we set mice in an accelerated speed program from 4–40 rpm for 5 min. We measured the latency to the first fall. For the elevated plus maze test, we allowed mice (n = 10–17) to explore the maze for 5 min while being videotaped. We counted one entry only when all four paws were inside the arm and each entry to the center when the mouse left a particular arm.

Light/dark choice test. The light/dark choice test was performed as described previously[47].

Novel object. The novel object test was performed as described elsewhere[48]. Briefly, we habituated mice for 30 min for 2 consecutive days to 25 cm × 25 cm × 25 cm boxes. On the third day, we placed them in the same boxes for 10 min in the presence of two identical plastic tubes. An hour later, we returned them to the cages and we replaced one of the tubes with a rubber ball of similar size. We measured and plotted the number of times the mouse approached the novel object (head toward the ball and distance less than 2 cm).

Conditioned place preference. We subjected mice to 4 d of conditioning sessions as described previously[49] using cocaine at doses of 5 or 0.5 mg per kg. Previous to conditioning (pretest) mice of both genotypes did not show floor preferences in a paired t test analysis (grid versus floor) as a group. Given that each individual mouse showed a slight preference for one floor type, they were all conditioned to the other floor type. We considered animals to be conditioned if the time spent on the drug-paired floor increased significantly after conditioning in comparison with time on that same floor during pretest (30 min). We analyzed results using repeated-measures ANOVA.

Continuous reinforcement task. We performed experimental conditions and initial training as described previously[50], but without lever retraction. We maintained mice at 85% of their normal body weight and trained them in an FR1 schedule (one pellet for one press) for 5 d. Finally, we set mice in FR3, FR10, FR30, FR100 food self-administration procedures for 3 d each except the FR100 task that was performed once. During these schedules, we set the time cutoff at 3 min, after which the lever retracted and program ended. Data are averages of the last 2 d ± s.e.m. of the number of pellets obtained (even

though no differences were found between the first and following days) and were analyzed using repeated-measures ANOVA.

Progressive ratio task. We trained mice as described above. Then, we trained mice for 2 weeks on a progressive ratio schedule where they had to press a lever in a progressive manner (2^n) to get successive 20-mg pellets in a 3-min cutoff. We measured the number of pellets obtained and break point (defined as the last series in which a pellet was obtained), number of presses and session time. For statistical analysis, we performed repeated-measures ANOVA, using data from the last 5 d. Extinction schedule: we placed mice in the operant chambers for the two following days in a 60-min extinction program in which no food was delivered.

Statistical analysis. We analyzed data using an ANOVA, and repeated measures where appropriate. Significant ANOVAs were followed up with Fisher LSD test.

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