Supporting Information

Li et al. 10.1073/pnas.1109718108

SI Materials and Methods

Electrophysiological Recoding in Prefrontal Cortical Slices. Transgenic mice were generated at Columbia University according to the methods described previously (1). Most of the data were obtained from young adult mice (3–4 mo) except those with doxycycline-supplemented chow (2 mo). Briefly, mice expressing the human D2 receptor under the tetO minimal promoter were generated on a C57BL/6-CBA(J) F2 background. TetO mice were backcrossed for over 10 generations to the C57BL/6(J) background and then crossed for electrophysiological studies with mice expressing the tTA transgene under the CamKIIα promoter (2). CamKII-tTA mice were on 129SveV(Tac)N20-22 background. Only F1s between backcrossed animals were used for the electrophysiological analysis. Offspring were genotyped by PCR for tTA and tet-O transgenes (3). Double transgenic mice (D2R-OE mice), and control littermates (tetO, tTA or control animals) were used for electrophysiological recordings. For regulating tetO-driven expression, mice were fed doxycycline-supplemented chow (40 mg/kg, Mutual Pharmaceutical). The animals were treated under the guidelines of the National Institutes of Health and the experimental protocol was approved by the Institutional Animal Care and Use Committee at Drexel University College of Medicine.

The detailed procedures for physiological recordings can be found in our previous publications (4–6). The animals were deeply anesthetized with Euthasol (0.2 mL/kg, Virbac) and euthanized by decapitation. Their brains were immediately removed and placed in ice-cold (<4 °C) sucrose solution. The solution contains the following ingredients (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 7.0 $MgSO_4$, 213 sucrose; pH 7.4. The solution was buffered with 95% O_2 and 5% CO_2. The blocks of neocortex containing medial PFC (PrL, Paxinos and Watson, The Rat Brain in Stereotaxic Coordinates. 2005) were trimmed and sliced into 300-μm sections using a Leica VT1000s Vibratome (Leica Microsystems). The brain slices were incubated in oxygenated Ringer's solution at 35 °C for 1 h. Ringer's solution contains the following ingredients (in mM): 128 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgSO_4$, 26 $NaHCO_3$, and 10 dextrose, pH 7.4. The slices were kept at room temperature until being transferred to a submerged chamber for recording. Whole-cell patch clamp recordings were conducted in the medial PFC through an upright Zeiss Axioskop 2 microscope (Carl Zeiss) at ∼35 °C. Resistance of the recording pipette (1.2 mm borosilicate glass, Warner Instrument) was 7–9 MΩ.

A Cs^+-based intracellular solution was loaded into recording pipettes to block both sodium and potassium channels and used for excitatory postsynaptic current (EPSC) recordings. The Cs^+ solution contained (in mM) Cs^+-gluconate 120, QX-314 5, CsCl2 6, ATP-Mg 1, Na2GTP 0.2, Hepes 10, and 0.3% biocytin at pH 7.3. The layer V pyramidal neurons were recorded in the voltage-clamp mode with membrane potentials held at -70 mV in the presence of picrotoxin (50 μM) for spontaneous EPSCs, or with both picrotoxin and tetrodotoxin (TTX, 0.5 μM) for miniature EPSCs (mEPSCs). The evoked EPSCs were elicited by stimulating II/III with a 10-pulse 20-Hz train (0.1 ms, 10–100 μA, 0.1 Hz) through a bipolar electrode placed ∼200–500 μm from the recording neurons. Similarly, the spontaneous inhibitory postsynaptic currents (sIPSC) and miniature IPSCs (mIPSCs) at the layer V pyramidal neurons were recorded at -70 mV in the presence of D-AP5 (50 μM) and NBQX (10 μM) to block glutamatergic receptors without or with tetrodotoxin (0.5 μM), respectively. A high chloride Cs+-based intracellular solution (134 mM CsCl2, 2 mM MgCl2, 2 mM Na2-ATP, 0.5 mM Na2GTP, 5 mM Na2-phosphocreatine, 1 mM EGTA, 10 mM Hepes, and 0.3% biocytin, pH 7.25) was used for IPSC recordings. Because we used high chloride Cs+-based intracellular solution (134 CsCl) to record IPSC, the GABAa mediated currents showed as inward currents when membrane potential was hold at -70 mV because the reversal potential of Cl- channel under such a recording condition is about +2 mV. The evoked IPSCs were elicited by stimulating layer II/III with a bipolar electrode placed ∼300 μm from the recording neurons (10 pulses at 20 Hz; 0.1 ms, 10–100 μA, 0.1 Hz). In another set of experiments, the action potentials of the layer V pyramidal neurons were recorded in current-clamp mode and the recording pipettes were filled with a K^+-based intracellular solution containing (in mM) 120 K-gluconate, 6 KCl, 0.5 $CaCl_2$, 0.2 EGTA, 4 ATP-Mg, 10 Hepes, and 0.3% biocytin, with a final pH of 7.25.

Data Analysis. The spontaneous and miniature EPSCs and IPSCs recorded in voltage-clamp mode were analyzed with Clampfit 9.2 (Axon Instruments). A typical s/mEPSC or s/mIPSC at each recording condition was selected to create a sample template for the event detections within a data period. The frequency (number of events) and amplitude of the individual events were examined with the threshold set at the medium level (i.e., 5–6) in Clampfit. The amplitudes of the evoked EPSCs and IPSCs were measured by averaging 30 traces from the onset to the peak of the EPSC with Clampfit 9.2 software. Only the neurons that produced stable baseline EPSCs or IPSCs without rundown for at least 5 min were used for further analysis of drug and stimulus effects. The data were analyzed with the Student t test for statistical significance with 95% confidence and were presented as mean ± SE. The numbers of neurons (n) in each group were derived from 3 to 5 mice.

SI Results

To examine the interactions between DA applications and PPRs, we used two-way ANOVA and found no significant differences in all concentrations applied ($F = 0.05$, $P = 1.00$, 1 μM DA in control mice; $F = 0.10$, $P = 1.00$, 1 μM DA in D2-OE mice; $F = 0.04$, $P = 1.00$, 10 μM DA in control mice; $F = 0.15$, $P = 1.00$, 10 μM DA in D2-OE mice; $F = 0.38$, $P = 0.93$, 100 μM DA in control mice; $F = 0.28$, $P = 0.97$, 100 μM DA in D2-OE mice; Fig. 3*B*).

SI Discussion

The striatal mediums spiny neurons (MSNs) can be divided in two populations based on their projections fields. In the dorsal striatum striato-nigral MSNs project monosynaptically to the substantia nigra and the entopeduncular nucleus (iGP in primates) whereas striato-pallidal MSNs project to the lateral globus pallidus (eGP in primates) which in turn projects to the substantia nigra (7, 8). In addition to their monosynaptic projections striato-nigral projections possess limited collateral terminal fields in the lateral globus pallidus (9).

The two striatal MSN populations can further be distinguished by their gene expression profile (10). The expression of D1Rs and D2Rs is largely divided between striato-nigral and striato-pallidal MSNs and in the dorsal striatum the percentage of neurons that express both receptors is in the range of 5–8% (11–13), whereas in the ventral striatum the percentage coexpression is higher with levels up to 33% (11, 13). In D2R-OE mice the percentage of neurons that express both receptors in the dorsal striatum is increased to around 22% because the CamKIIalpha

promoter used for transgene expression doesn't distinguish striato-nigral and striato-pallidal projections. Which specific neurons mediate the increase in D2R binding potential observed in schizophrenia is unknown. However, a recent finding found increased heterodimerization between D1Rs and D2Rs in patients with schizophrenia (13) suggesting that the percentage of neurons coexpressing both receptors could be increased in schizophrenia.

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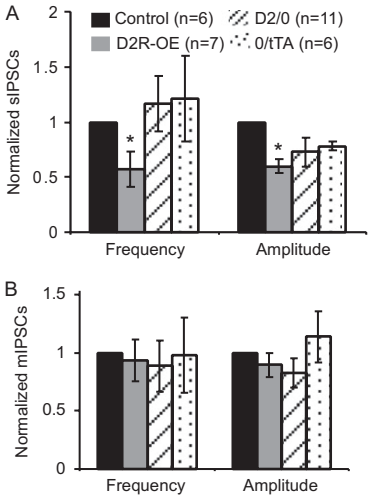

Fig. S1. Overexpression of D2 receptors in the striatum results in decreases in both frequency and amplitude of sIPSCs in PFC layer V pyramidal neurons compared with other control mice. (*A*) Summary histogram showing the sIPSC frequency and amplitude recorded from the mice of different genotypes. Overall analysis with ANOVA also demonstrated significant decreases of sIPSC frequency and amplitude in D2R-OE mice when compared among the four groups (P = 0.018 for frequency and P = 0.025 for amplitude). Post hoc analyses indicated that although the sIPSC amplitudes in both D2/0 and 0/tTA seemed to be decreased, the differences were not significant (control vs. D2/0 mice, P = 0.241; control vs. 0/tTA mice, P = 0.256). There were no statistical differences between D2R-OE and D2/0 mice (P = 0.471) but there was a significant difference between D2R-OE and 0/tTA mice (P = 0.041) and D2R-OE and control mice (P = 0.044). (*B*) The mIPSCs in all mice genotypes seem to be similar without any statistical difference (n = 5 in each genotype, $P > 0.05$ for all).