Altered Corticostriatal Neurotransmission and Modulation in Dopamine Transporter Knock-Down Mice

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Wu N, Cepeda C, Zhuang X, Levine MS. Altered corticostriatal neurotransmission and modulation in dopamine transporter knock-down mice. J Neurophysiol 98: 423–432, 2007. First published May 23, 2007; doi:10.1152/jn.00971.2006. Dopamine (DA) modulates glutamate neurotransmission in the striatum. Abnormal DA modulation has been implicated in neurological and psychiatric disorders. The development of DA transporter knock-down (DAT-KD) mice has permitted modeling of these disorders and has shed new light on DA modulation. DAT-KD mice exhibit increased extracellular DA, hyperactivity, and alterations in habituation. We used whole cell patch-clamp recordings from visually identified striatal neurons in slices to examine the effects of DAT-KD on corticostriatal transmission. Electrophysiological recordings from medium-sized spiny neurons in the dorsal striatum revealed alterations in both amplitude and frequency, of spontaneous glutamate receptor-mediated synaptic currents in cells from DAT-KD mice. Furthermore, kinetic analyses revealed that these currents had shorter half-amplitude durations and faster decay times. In contrast, GABA-receptor-mediated synaptic currents were not altered. Striatal neurons from DAT-KD mice also responded differently to amphetamine, cocaine, and DA D2-receptor agonists or antagonists compared with wildtype (WT) lintermate controls. In WT's amphetamine and cocaine reduced the frequency of spontaneous glutamate currents and these effects appeared to be mediated by activation of D2 receptors. In contrast, in DAT-KD mice either no changes or only small increases in frequency occurred. D2-receptor agonists or antagonists also had opposing effects in WT and DAT-KD mice. Together, these results indicate that chronically increased extracellular DA produces long-lasting changes in corticostriatal communication that may be mediated by changes in D2-receptor function. These findings have implications for understanding mechanisms underlying attention deficit hyperactivity disorder and Tourette’s syndrome and may provide insights into novel therapeutic approaches.

INTRODUCTION

Abnormal dopamine (DA) transmission in the basal ganglia and cerebral cortex has been implicated in a number of neurological and psychiatric disorders such as Parkinson’s disease, schizophrenia, Tourette’s syndrome, and attention deficit hyperactivity disorder (ADHD) (reviewed in Albin and Mink 2006; Nieoullon and Coquerel 2003; Seamans and Yang 2004; Swanson et al. 1998). The DA transporter (DAT), a membrane protein that recycles released DA into presynaptic terminals (Jones et al. 1998a), plays a key role in altered DA transmission. Genetic studies in humans have demonstrated a close linkage of the DAT gene and ADHD (Cook et al. 1995; Gill et al. 1997; Waldman et al. 1998). DAT is also the target of psychostimulants, a common treatment for ADHD (reviewed in Madras et al. 2005).

The generation of mice with targeted deletion of the DAT gene has allowed a better understanding of DAT function (reviewed in Chen and Zhuang 2003). DAT knock-out mice are hyperdopaminergic and display hyperactivity and memory deficits (Giros et al. 1996). However, these animals also show growth defects and pituitary hypoplasia (Bosse et al. 1997). More recently, a DAT knock-down (KD) mouse with decreased expression of DAT [~10% of wildtype (WT) level] has been developed (Zhuang et al. 2001). These mice are also hyperdopaminergic and hyperactive and display enhanced motivation for food reward (Cagniard et al. 2006; Pecina et al. 2003; Yin et al. 2006). However, they do not show growth abnormalities (Zhuang et al. 2001). These attributes make the DAT-KD mice a potentially more suitable animal model of ADHD.

Several mouse models of basal ganglia diseases show alterations in synaptic transmission along the corticostriatal pathway. For example, we have shown reduced spontaneous and evoked glutamate synaptic activity in mouse models of Huntington’s disease (Cepeda et al. 2003; Klapstein et al. 2001). Some similar changes were observed in a mouse model of Parkinson’s disease (Goldberg et al. 2003). Interestingly, in these mouse models DA transmission is also significantly altered (Goldberg et al. 2003; Johnson et al. 2006).

The aim of the present study was to examine potential alterations in spontaneous excitatory synaptic currents (sEPSCs) in striatal medium-sized spiny neurons (MSSNs) in DAT-KD mice. Because DA modulates responses evoked by activation of glutamate receptors (Cepeda et al. 1993), we hypothesized that abnormal chronic exposure to high levels of DA would produce alterations in synaptic activity along the corticostriatal pathway. We also examined the effects of drugs that alter DA function, some of which are used to treat ADHD.

METHODS

All experimental procedures were carried out in accordance with the USPHS Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at UCLA. Experiments were conducted on DAT-KD (n = 35) and age-matched lintermate controls (WT, n = 25). Average ages were 69 ± 1.7 and 68 ± 2.1 days for DAT-KD and WT animals, respectively, and did not differ between the two groups.

After sacrificing the brains were dissected and immediately placed in oxygenated ice-cold low-calcium artificial cerebrospinal fluid
was compensated 70–80% and checked periodically. If MSSNs were identified by somatic size, basic membrane properties with the aid of infrared videomicroscopy (Cepeda et al. 1998a). Voltage-clamp mode were obtained from MSSNs visualized in slices flowing ACSF (4 ml/min). Whole cell patch-clamp recordings in voltage-clamp mode by applying a depolarizing step-voltage command (10 mV) and using the membrane test function integrated in the pClamp8 software (Axon Instruments, Foster City, CA). This function reports membrane capacitance (in pF), input resistance (in MΩ), and time constant (in ms). The time constant was obtained from a single exponential fit to the decay of the capacitive transients. In addition, a separate group of neurons from DAT-KD and WT mice were recorded in current-clamp mode using patch electrodes containing (in mM): K-glutamate 140, HEPES 10, MgCl2 2, CaCl2 0.1, EGTA 1.1, and K2 ATP 2 (pH 7.25–7.3, osmolality 280–290 mOsm). Resting membrane potential (RMP), action potential parameters, and responses to depolarizing and hyperpolarizing square-wave current pulses were examined.

Spontaneous postsynaptic currents were recorded in standard ACSF composed of the following (in mM): NaCl 130, NaHCO3 26, KCl 3, MgCl2 2, NaHPO4 1.25, CaCl2 2, glucose 10 (pH 7.2–7.4, osmolality 290–300 mOsm). Cells were held at −70 mV to minimize the contribution of γ-aminobutyric acid type A (GABA_A) receptors and that of voltage-gated conductances. In addition, in most experiments bicuculline methiodide (BIC, 10 μM; Tocris, Ellisville, MO) was added to block the contribution of spontaneous currents mediated by activation of GABA_A receptors. After characterizing the basic membrane properties of the neuron, sEPSCs were recorded for variable periods of time (usually 3–6 min). The membrane current was filtered at 1 kHz and digitized at 200 μs using Clampex (gap-free mode; Axon Instruments). In some cells tetrodotoxin (TTX, 1 μM; CalBiochem, La Jolla, CA) was added to isolate miniature (m)EPSCs, events that were not dependent on presynaptic action potentials.

Drugs affecting DA-receptor function, d-amphetamine (25 μM), cocaine (10 μM), quinpirole (10 μM), sulpiride (10 μM), and R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-1,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390, 20 μM, a D1-receptor antagonist), or acetylcholine receptor function, atropine sulfate (1 μM, a muscarinic antagonist) and muscarine (10 μM) were purchased from Sigma–Aldrich (St. Louis, MO). They were prepared as stock solutions in double-distilled water and diluted to their final concentration before each experiment. Sulpiride was freshly made for each experiment. All drugs were applied in the bath.

Spontaneous synaptic events were analyzed off-line using the Mini Analysis Program (Jaejin Software, Leonia, NJ). This software was used to calculate EPSC frequency and amplitude of events and to construct amplitude–frequency histograms. The threshold amplitude for the detection of an event was adjusted to at least twofold above the root-mean-square noise level [generally 2–3 pA at −70 mV, 4–5 pA at +20 mV, and about 10 pA when 4-aminopyridine (4-AP, Sigma–Aldrich) was in the bath]. Frequencies were expressed as number of events per second (Hz). EPSC kinetic analysis was performed using the Mini Analysis Program. Events with peak amplitudes between 6 and 50 pA were grouped, aligned by half-rise time, and averaged to obtain rise times, decay times, and durations at half-amplitude. Events with complex peaks were eliminated from this analysis. First- and second-order exponential curves were fit with a maximum of 5,000 iterations. Values in the figures and text are presented as means ± SE. Differences in mean currents at various holding voltages were assessed with two-way ANOVAs with one repeated measure followed by multiple comparisons using Bonferroni t-test. Student’s t-tests alone were used when only two group means were compared. Differences between means were considered statistically significant if P < 0.05.

RESULTS

Based on somatic size and typical membrane properties (Cepeda et al. 1998a) all recorded cells were MSSNs. The identity of a subset of cells was further confirmed after histological processing for biocytin (n = 20, ten in each group). The passive membrane properties of MSSNs recorded in voltage-clamp mode (Cs-methanesulfonate in the patch pipette) were similar in both groups [cell capacitances were 81 ± 2.9 pF in WT (n = 68 cells) and 80 ± 2.6 pF in DAT-KD (n = 79 cells); input resistances were 71 ± 2.9 MΩ in WT and 79 ± 4.7 MΩ in DAT-KD; time constants were 1.36 ± 0.05 ms in WT and 1.34 ± 0.04 ms in DAT-KD]. In the separate group of neurons (n = 16 in DAT-KD and n = 13 in WT) recorded in current-clamp mode (K-glutamate in the patch pipette), RMPs and action potential properties were examined. RMPs (−78 ± 1.2 mV in DAT-KD and −78.3 ± 1.2 mV in WT), action potential parameters (amplitude 76.6 ± 1.8 mV in DAT-KD and 78.2 ± 2.3 mV in WT; rise time 0.68 ± 0.02 ms in DAT-KD and 0.69 ± 0.03 ms in WT; decay time 2.1 ± 0.13 ms in DAT-KD and 1.84 ± 0.08 ms in WT; half-amplitude duration 1.97 ± 0.07 ms in DAT-KD and 1.96 ± 0.07 ms in WT), action potential thresholds (−49.6 ± 1.4 mV in DAT-KD and −49.0 ± 1.1 mV in WT), and responses to intracellular injections of square-wave current pulses were similar in neurons from DAT-KD and WT animals (Fig. 1). All cells recorded demonstrated inward rectification in the hyperpolarizing direction, a defining feature of MSSNs (Fig. 1).

DAT-KD mice exhibit a greater frequency of intermediate-amplitude sEPSCs

Spontaneous synaptic currents were recorded in voltage clamp (Cs-methanesulfonate in the patch pipette) at a holding potential of −70 mV in standard ACSF (Fig. 2A). At this potential all spontaneous currents were inward and mostly reflected non-N-methyl-d-aspartate (NMDA) receptor activation by glutamate. The frequency of these currents was not significantly changed by BIC (Fig. 2B). Application of the non–NMDA-receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) in a subset of recorded neurons (n = 30) completely blocked spontaneous synaptic currents, indicating they were mediated predominantly by activation of α-amino-3-hydroxy-5-methyl-4-isoxazoloproprionic acid (AMPA)/kainate receptors (Fig. 2C). The mean frequency of spontaneous synaptic currents was 2.06 ± 0.13
WT and DAT-KD mice.

The presence of BIC (10 μM) significantly reduced (10–20%) glutamate receptor-mediated spontaneous excitatory postsynaptic currents (sEPSCs) were isolated by bath applying bicuculline methiodide (BIC, 10 μM). C: sEPSCs were blocked by 6-cyano-7-nitroquinolinic acid-2,3-dione (CNQX, 10 μM), a non-N-methyl-D-aspartate (NMDA) receptor antagonist, demonstrating they were mediated by non-NMDA ionotropic glutamate receptors. D: BIC produced a small reduction in the frequency of sEPSCs in both WT and DAT-KD mice. E: amplitude–frequency histograms of sEPSCs in ACSF. F: amplitude–frequency histograms of sEPSCs in BIC. G: cumulative distribution of events in the different amplitude bins in ACSF and in the presence of BIC (H). Asterisks in each panel indicate mean frequencies for WT and DAT-KD mice.

FIG. 2. A: spontaneous synaptic currents were recorded in standard artificial cerebrospinal fluid [ACSF; holding potential (Vh) = −70 mV]. B: glutamate receptor-mediated spontaneous excitatory postsynaptic currents (sEPSCs) were isolated by bath applying bicuculline methiodide (BIC, 10 μM). C: sEPSCs were blocked by 6-cyano-7-nitroquinolinic acid-2,3-dione (CNQX, 10 μM), a non-N-methyl-D-aspartate (NMDA) receptor antagonist, demonstrating they were mediated by non-NMDA ionotropic glutamate receptors. D: BIC produced a small reduction in the frequency of sEPSCs in both WT and DAT-KD mice. E: amplitude–frequency histograms of sEPSCs in ACSF. F: amplitude–frequency histograms of sEPSCs in BIC. G: cumulative distribution of events in the different amplitude bins in ACSF and in the presence of BIC (H). Asterisks in each panel indicate mean frequencies for WT and DAT-KD mice.

Because at a holding potential of −70 mV, GABA A-receptor–mediated currents may also contribute to the total number of spontaneous synaptic currents (Cepeda et al. 2003), we examined the frequencies of spontaneous synaptic currents in the presence of BIC (10 μM), a GABA A-receptor antagonist. BIC produced either no change or a small reduction (10–20%) in the frequency of events (Fig. 2, B and D, right) when compared with standard ACSF in neurons from WT and DAT-KD mice. The frequency of spontaneous inward currents in the presence of BIC in DAT-KD mice was 2.10 ± 0.18 Hz and in WTs it was 1.88 ± 0.12 Hz; the difference was not statistically significant (Fig. 2D, right). Amplitude–frequency histograms indicated that DAT-KD mice exhibited significantly fewer events in the 6- to 8-pA range (P < 0.05; Fig. 2F) and significantly more events in the range of 12–18 pA in the presence of BIC (P values <0.05–0.01; Fig. 2F).}

Overall, these results suggest that the presence of more intermediate-amplitude events in cells from DAT-KD mice may have contributed to differences in kinetics. It is also possible that in striatal neurons from DAT-KD mice there were changes in subunit composition of postsynaptic glutamate receptors and/or faster glutamate clearance from the synaptic cleft.

FIG. 1. Responses induced by injection of hyperpolarizing and depolarizing current pulses in medium-sized spiny neurons (MSSNs) from wildtype (WT) and dopamine transporter knock-down (DAT-KD) mice. Cells were recorded with patch pipettes (K-glucuronate as the internal solution) in current-clamp mode. Resting membrane potentials (RMPs) and action potential parameters were similar in cells from both groups of mice (see text for details). Graph at the right shows similar average current–voltage relationships in MSSNs from WT and DAT-KD mice.
Larger-amplitude currents in DAT-KD mice are dependent on action potentials

Because the presence of large synaptic events could be a function of increased firing of presynaptic neurons or of increased multiquantal release, we next tested whether presynaptic cell firing contributed to increases in the amplitude of spontaneous synaptic activity by applying BIC to block GABA\(_A\)-receptor–mediated currents and TTX (1 \(\mu\)M) to block the activation of voltage-gated Na\(^+\) channels and to determine the percentage of spontaneous synaptic currents that are independent of action potentials (mEPSCs). Neurons were held at 70 mV. TTX significantly decreased the frequency of spontaneous postsynaptic currents in all recorded MSSNs from WTs and DAT-KDs (Fig. 4, A–C). The mean frequencies of mEPSCs after TTX were not significantly different (1.28 ± 0.23 Hz (\(n = 9\)) vs. 1.52 ± 0.17 Hz (\(n = 24\)), in cells from WTs and DAT-KDs, respectively). About 80% of sEPSCs in WTs and about 75% in DAT-KDs were TTX resistant (Fig. 4D). Although there were significant differences between the cumulative amplitude histograms from WT and DA-KD cells before TTX (\(P\) values <0.05–0.01, 6- to 12-pA-amplitude bins; Fig. 4F), after TTX these differences no longer occurred (Fig. 4F, right graph). Cumulative frequency distributions of interevent interval (a measure of release probability) demonstrated that the shift to the left, indicative of proportionately more shorter interevent intervals in cells from DAT-KD mice, was reduced in the presence of TTX (Fig. 4G). Because the overall frequency of mEPSCs was not different, larger-amplitude events in DAT-KD mice were likely dependent on action potential generation. Interestingly, the kinetic properties of mEPSCs were also similar in cells from WT and DAT-KD mice (Fig. 4E), suggesting that the faster kinetic properties observed in the absence of TTX arose primarily from the occurrence of larger synaptic currents.
Cholinergic interneurons are not responsible for the increased frequency of intermediate-amplitude currents in DAT-KD mice

To examine the possibility that differential tonic firing of cholinergic interneurons may have contributed to the differences in sEPSCs in MSSNs from DAT-KD and WT mice, we recorded the activity of visually and electrophysiologically identified large, putative cholinergic interneurons (n = 4 in WT and n = 5 in DAT-KD). These interneurons have been shown to fire spontaneously in slices (Bennet and Wilson 1999). Indeed, in our recording conditions we confirmed spontaneous firing. However, no difference in firing rates was observed (2.5 ± 0.7 Hz in WT and 2.4 ± 0.5 Hz in DAT-KD). We also examined the effects of muscarine (10 μM, n = 9 and 11 cells from WT and DAT-KD mice, respectively) or the muscarinic antagonist atropine (1 μM, n = 11 and 12 cells from WT and DAT-KD mice, respectively) on the frequency of sEPSCs in MSSNs from WT and DAT-KD mice. Muscarine produced almost no change in frequency (−4 ± 4.6% change in WT vs. −4 ± 6.0% change in DAT-KD cells). Atropine produced a small nonstatistically significant decrease in both groups (−7 ± 5% change in WT vs. −13 ± 6% change in DAT-KD cells). Thus cholinergic inputs have a relatively minor contribution to the observed changes in sEPSCs.

The amplitude but not the frequency of spontaneous NMDA currents is changed in DAT-KD mice

One next question was whether a difference in NMDA-receptor–mediated currents between the two groups also existed. To answer this we isolated NMDA currents by modifying the ACSF solution. First Mg²⁺ was eliminated to remove the voltage dependency of activation of NMDA receptors and then BIC (10 μM) was added to block GABA_A-receptor–mediated currents. As illustrated in Fig. 5, A and E (left), the frequency of sEPSCs in both groups was about the same in this external solution [1.52 ± 0.21 Hz in WT cells (n = 13) and 1.47 ± 0.14 Hz in DAT-KD cells (n = 18)]. However, the amplitude–frequency histograms revealed that neurons from DAT-KD mice had a significantly lower frequency of small-amplitude events (8–12 and 12–16 pA, P < 0.01; Fig. 5E, middle and right). In contrast, compared with WTs, cells from DAT-KD mice exhibited a higher frequency of larger-amplitude spontaneous synaptic currents (>20 pA) in Mg²⁺-free ASCF and BIC (P < 0.05; Fig. 5E, middle and right). The fact that CNQX (10 μM) blocked almost all spontaneous activity (Fig. 5B) demonstrated that, in spite of removal of Mg²⁺, most of these currents were still mediated by activation of non-NMDA receptors.

Because omission of Mg²⁺ from the external ACSF solution was not sufficient to induce spontaneous NMDA-receptor–mediated currents 4-AP (100 μM), a K⁺ channel blocker that enhances neurotransmitter release was added (Cepeda et al. 2001; Flores-Hernández et al. 1994). In Mg²⁺-free ASCF and in the presence of CNQX and BIC, application of 4-AP induced large-amplitude inward currents in MSSNs from WT (n = 13) and DAT-KD (n = 17) mice (Fig. 5C) that were completely abolished by bath application of 2-amino-5-phosphonovalerate (AP-5, 50 μM), an NMDA-receptor antagonist (Fig. 5D). The average frequency of NMDA currents induced by 4-AP was not significantly different between groups (1.99 ± 0.34 Hz in WTs and 1.59 ± 0.28 Hz in DAT-KDs; P > 0.05; Fig. 5F, left). Amplitude–frequency histograms demonstrated that the slightly lower average frequency in neurons from DAT-KDs was the result of a significantly reduced frequency of smaller-amplitude events (20–30 pA, P < 0.03; Fig. 5F, right). In contrast, the frequency of large-amplitude NMDA events (>40 pA) induced by 4-AP application did not differ between cells from WTs and DAT-KDs (Fig. 5F, right). This may be explained by the fact that 4-AP produced a generalized increase in large-amplitude events in both groups of mice.

Spontaneous GABAergic synaptic activity is not altered in DAT-KD mice

GABA is the principal inhibitory neurotransmitter in the basal ganglia and is the transmitter used by most neurons of the
striatum, including the MSSNs and a variety of inhibitory interneurons (Czubayko and Plenz 2002; Koos and Tepper 1999; Oertel and Mugnaini 1984; Venance et al. 2004). Therefore we also examined spontaneous currents mediated by activation of GABA<sub>A</sub> receptors in neurons from DAT-KD mice. In standard ACSF cells were held at +20 mV to increase the driving force of GABA<sub>A</sub> synaptic currents (in our experimental conditions the equilibrium potential for GABA<sub>A</sub> currents is around ~60 mV). At this holding potential, spontaneous synaptic currents were outward (Fig. 6A) and glutamate currents did not contribute because application of BIC (10 μM) completely abolished all synaptic activity (Fig. 6B). WT and DAT-KD cells exhibited approximately equal mean frequencies of spontaneous GABA<sub>A</sub>-receptor–mediated outward currents [Fig. 6C, 3.25 ± 0.6 Hz in WTs (n = 13) and 3.66 ± 1.0 Hz in DAT-KDs (n = 15)]. The difference in mean frequencies between WT and DAT-KD animals was not statistically significant. Amplitude–frequency histograms showed that all cells from DAT-KD mice exhibited small increases in frequency of events at all amplitudes but again these increases were not statistically significant (Fig. 6D). These results indicate that DAT-KD mainly affects glutamate-receptor–mediated currents.

Effects of amphetamine and cocaine

DAT-KD mice express only about 10% of the DAT compared with WT levels and their ability to clear released DA decreases, which results in elevated DA tissue levels (~50%) as measured by in vivo microdialysis (Zhuang et al. 2001). To examine whether the high extracellular DA concentration would affect the sensitivity to pharmacological manipulations of the DA system in DAT-KD mice, we bath-applied amphetamine (APT, 25 μM), an indirect agonist that increases DA release and prevents DA reuptake (Jones et al. 1998b). The data examining effects of APT [as well as cocaine (COC), quinpirole (QUIN), and sulpiride (SULP)] were analyzed with a two-way ANOVA and the interaction term between genotype and drug application was statistically significant. Subsequent changes in frequency were assessed with post hoc t-test (Bonferroni). After applying APT, the frequency of EPSCs in MSSNs was significantly reduced in WT cells [26%, F(genotype × drug interaction) = 4.74, df = 1/32, P = 0.037, n = 11 WT cells and n = 20 DAT-KD cells; t = 2.34, P = 0.024; Fig. 7, A and B]. The percentage increase in DAT-KD cells (10%) was not statistically significant. APT application did not affect the amplitude of the spontaneous currents.

COC increases DA availability in the striatum mainly through the blockade of transporter-mediated reuptake (Centonze et al. 2002). Therefore we hypothesized that the effect of COC on sEPSCs in MSSNs from DAT-KDs should be reduced. In the presence of BIC, COC (10 μM) induced a statistically significant decrease in the frequency of spontaneous synaptic currents in WTs [20%, F(genotype × drug interaction) = 5.95, df = 1/29, P = 0.021, n = 16 WT cells and n = 15 DAT-KD cells; t = 2.78, P = 0.019; Fig. 7, C and D]. The small increase in DAT-KD cells (7%) was not statistically significant. COC application also did not affect the amplitude of the spontaneous currents. Taken together, these results suggest that in DAT-KD mice the ability of DA to modulate glutamate release at the corticostriatal pathway is impaired.

Role of DA receptors

One possible explanation for the changes in sEPSCs in WTs is that the increase in DA release caused by APT and COC activated presynaptic D2 receptors, which can attenuate glutamate release (Kornhuber and Kornhuber 1986; Rowlands and Roberts 1980; Yamamoto and Davy 1992), therefore reducing the frequency of sEPSCs (Cepeda et al. 2001; Flores-Herrán-
and BIC (10 μM) in WT cells. Under these conditions APT and COC no longer produced significant decreases in the frequency of sEPSCs (APT: −12 ± 9%, n = 5; COC: −8 ± 15%, n = 5). SULP also blocked the effects of QUIN (−7 ± 11%, n = 5). Thus the effects of APT and COC were mediated by activation of D2 receptors.

Finally, we also examined the role of D1 receptors in DAT-KD mice. SCH 23390 (20 μM), a D1-receptor antagonist, had minimal effects on the frequency of sEPSCs (not shown). We then applied APT (25 μM) to see whether blockade of D1 receptors affected DA modulation of sEPSCs. In the presence of SCH 23390, APT decreased the frequency of sEPSCs in cells from WT mice but had practically no effect in cells from DAT-KDs (WTs: −21%, n = 10; DAT-KDs: −2%, n = 16). The results suggest that D1 receptors have a minimal role in the modulation of EPSCs frequency in conditions of hyperdopaminergia.

**DISCUSSION**

The present study demonstrated a series of changes in spontaneous glutamatergic synaptic activity in the striatum of DAT-KD mice. In contrast, GABAergic synaptic activity did not appear to be affected by the mutation. Although the overall frequency of sEPSCs in MSSNs was similar in DAT-KD and WT mice, the frequency of intermediate-amplitude events was significantly increased in cells from DAT-KD mice. Alterations in the kinetic properties of EPSCs also were observed. Furthermore, drugs that manipulate the DA system like APT and COC had minimal effects on sEPSC frequency in DAT-KD mice. Finally, the ability of a DA D2-receptor agonist and antagonist to modulate excitatory transmission along the corticostriatal pathway was severely compromised in DAT-KD animals, suggesting alterations in DA-receptor function. These deficits could be caused by downregulation of DA D2 receptors as the result of increased DA tone in mutant animals (Dumartin et al. 2000; Giros et al. 1996; Zhuang et al. 2001). The alterations are less likely explained by decreases in the levels of D2-receptor expression because this possibility was ruled out by a previous study (Zhuang et al. 2001).

It is well established that DA modulates glutamatergic transmission at the pre- and postsynaptic levels in striatum and cerebral cortex (Bamford et al. 2004b; Cepeda and Levine 1998; Cepeda et al. 1993, 1998, 2001; Flores-Hernández et al. 2002; Hernández-Echeagaray et al. 2004; Levine et al. 1996; Liu et al. 2004; Seamans and Yang 2004; Tseng and O'Donnell 2004). The direction of this modulation depends on the interactions of the glutamate and DA-receptor subtypes involved, as well as a number of other factors. Thus in general DA, by D1 receptors, enhances glutamate responses, particularly those mediated by activation of NMDA receptors. In contrast, by D2 receptors, DA decreases responses evoked by glutamate receptors, particularly those mediated by activation of AMPA/kainate receptors (Cepeda and Levine 1998; Cepeda et al. 1993). Other factors also affect DA modulation, such as concentration of DA, site of action at pre- or postsynaptic location of receptors, brain region, etc. (reviewed in Seamans and Yang 2004). In addition, genetic manipulations of DA receptors alter this modulation. For example, we demonstrated that mice lacking D1 receptors have a reduced capability to enhance NMDA responses (Levine et al. 1996) and mice with deletion...
of D4 receptors have increased cortical excitability (Rubinstein et al. 2001). Interestingly, mice with DA deficiency show morphologically normal corticostriatal terminals with hypersensitive D2 receptors (Bamford et al. 2004a).

Knock-down of DAT produces an elevation in tissue DA levels. DA release is decreased by about 25%, which is probably the result of deficient recycling, and clearance is appreciably decreased (Zhuang et al. 2001). Tissue DA levels are elevated by about 50% as measured by in vivo microdialysis. These changes in extracellular DA and its dynamics will have long-term effects on DA receptors, which may explain some of the electrophysiological alterations observed in the present study as well as the effects of DA-modulating drugs like APT and COC.

Striatal D2 receptors play a critical role in the modulation of glutamatergic activity in the corticostriatal pathway. These receptors are present pre- and postsynaptically (Tarazi and Baldessarini 1999; Wang and Pickel 2002) and exert a negative regulation of non-NMDA ionotropic glutamate receptor-mediated responses. At the postsynaptic level, we have shown that activation of D2 receptors decreases the amplitude of non-NMDA receptor-mediated responses (Cepeda et al. 1993; Hernández-Echeagaray et al. 2006). Although an early study suggested that the presynaptic modulation of glutamate transmission required a state of D2-receptor supersensitivity (Calabresi et al. 1992), others found that this modulation could occur in physiological conditions in a significant number of striatal neurons (Bamford et al. 2004b; Cepeda et al. 1994; Flores-Hernández et al. 1997; Hsu et al. 1995). In contrast, another study found clear modulation in the ventral but not in the dorsal striatum (Nicola and Malenka 1998). However, in this study very young animals were used and it is likely that effects were not seen because DA modulation in this region matures more slowly. It was recently shown that endocannabinoids mediate some of the presynaptic effects of D2-receptor activation (Kreitzer and Malenka 2005; Yin and Lovinger 2006). However, retrograde endocannabinoid modulation occurs only with strong, high-frequency stimulation (Yin and Lovinger 2006). In our recordings of sEPSCs it is less likely that endocannabinoid receptors play a significant role. Furthermore, animals with deletion of D2 receptors show facilitation of glutamate release at corticostriatal synapses, demonstrating a critical role of presynaptic D2 receptors (Cepeda et al. 2001).

Here we demonstrate that in striatal neurons from DAT-KD mice intermediate-amplitude sEPSCs occur more frequently in DAT-KD compared with WT animals and that some of these currents are dependent on action potentials. In conditions of chronic hyperdopaminergia there appears to be a marked alteration of presynaptic D2-receptor function. In normal conditions presynaptic D2 receptors serve as a filter of corticostriatal signaling by dampening glutamate release and reducing the occurrence of intermediate-amplitude synaptic events (Bamford et al. 2004b; Cepeda et al. 2001; Flores-Hernández et al. 1997). Reduced function of these receptors would allow more glutamate release along the corticostriatal pathway and proportionately more of these events occur and proportionately fewer small-amplitude events occur (6- to 10-pA bins). This down-regulation was also manifested by reduced effects of drugs that increase striatal DA concentration such as APT and COC. Finally, increased frequency of intermediate-amplitude events could have been further facilitated by downregulation of postsynaptic D2 receptors (Levine et al. 1996).

Alterations in the frequency of sEPSCs were less pronounced than changes in amplitude. This could be a consequence of expression of different DA-receptor isoforms. Thus it has been shown recently that the presynaptic modulation of glutamate transmission in the corticostriatal pathway depends on the D2 short isoform (Centonze et al. 2004). Interestingly, this study also showed that GABAergic transmission can be modulated by both isoforms, which could explain why we found no changes in GABAergic activity in DAT-KD mice.

NMDA-receptor–mediated responses were also affected. A shift in the amplitude–frequency distribution toward fewer small-amplitude events occurred in mutant mice. At present it is difficult to know what caused this shift. It is possible that the mutation also affected D1 receptors and this facilitated NMDA currents. However, because increased DA tone may also downregulate D1 receptors (Dumartin et al. 2000; Zhuang et al. 2001), this possibility seems unlikely. Another possibility is that downregulation of D2 receptors facilitates NMDA currents. We previously proposed that D2 receptors may function to counter excessive enhancement of NMDA responses by D1 receptors (Cepeda et al. 1998a,b; Flores-Hernández et al. 2002). If D2 receptors are downregulated in DAT-KD animals one would expect a facilitation of NMDA currents. It is important to note that a small number of DAT knockout mice demonstrate striatal cell death and some symptoms reminiscent of Huntington’s disease (Cyr et al. 2003). It is likely that an enhancement of glutamate-induced responses exacerbates the potentially toxic effects of DA. An additional possibility is that the shift away from small-amplitude sEPSCs reflects more frequent activation of postsynaptic receptors through action potential–dependent release as a consequence of downregulation of D2 receptors. One might speculate further that when Mg2+ is reduced cortical pyramidal neurons become hyperexcitable and may fire more action potentials. In fact, Mg2+ removal is a widely used model to induce epileptic discharges in hippocampus and cerebral cortex.

The electrophysiological alterations described here may be relevant to understand the mechanisms leading to behavioral and psychological disturbances in disorders in which an increase in DA function is hypothesized such as ADHD and Tourette’s syndrome. In fact, it could be speculated that motor hyperactivity arises from an alteration of glutamate currents. Reduced D2 function could leave the complement of corticostriatal signals unchecked, allowing for unfiltered inputs to reach the striatum. Attention could also be affected because increased glutamatergic function could translate into changes in motivational value as well as saliency of stimuli (Pecina et al. 2003). In hyperdopaminergic mice, everything would appear salient and deserving of attention. This means that therapeutic trials of drugs used to treat ADHD or Tourette’s syndrome not only should concentrate on the dysregulation of the DA system, but also should consider changes in other neurotransmitter systems. For example, it has been shown that the calming effect of psychostimulants in DAT-KO mice is not mediated by a reduction in DA levels, but by activation of the serotonergic system (Gainetdinov et al. 1999). It has also been shown that the hyperactivity of DAT-KO mice can be further enhanced by NMDA-receptor blockers. In contrast, drugs that increase glutamatergic transmission suppress the
hyperactivity of DAT-KO mice (Gainetdinov et al. 2001). A potential mechanism of these effects appears to involve modulation of serotonergic transmission in the frontal cortex (Gainetdinov et al. 2001; Sotnikova et al. 2006).

This implies that the final outcome of drugs used to treat disorders of DA function depends on their actions on multiple neurotransmitter systems affecting cortical neurons, the flow of information along the corticostriatal pathway, and the specific subsets of striatal neurons activated by those inputs. Evidently, more studies have to be performed to better understand these effects. Mouse models of hyperdopaminergia provide new ways to evaluate the mechanisms and potential benefits of drugs used to treat disorders caused by chronic increases of DA.

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