Altered function of glutamatergic cortico-striatal synapses causes output pathway abnormalities in a chronic model of parkinsonism

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Objective: In Parkinson’s disease, chronic striatal dopamine depletion results in over-activity and under-activity of the indirect and direct striatal output pathways respectively. In this study, we investigated changes in the function of glutamatergic cortico-striatal synapses that contribute to abnormalities in striatal efferents.

Methods: Whole-cell recordings were performed in striatal slices prepared from adult bacterial artificial chromosome mice, chronically lesioned with 6-hydroxydopamine (6-OHDA). Paired pulse facilitation, spontaneous synaptic activity, the ratio of AMPAR to NMDAR-mediated components of excitatory postsynaptic currents, AMPAR and NMDAR kinetics, current-voltage relationship and intrinsic membrane properties were assessed in indirect and direct pathway medium spiny neurons (MSNs), which were identified on the basis of expression of GFP, driven by the promoters of A2A or D1 receptor expression. The trajectory of striatal efferents, with respect to selective targeting of the globus pallidus and substantia nigra was also compared in sham-operated versus 6-OHDA-lesioned mice.

Results: Dopamine depletion did not affect the number of pathway specific output neurons or the trajectory of striatal outputs. In sham-operated animals, cortico-striatal synapses of both striatal efferent populations exhibited paired pulse facilitation and similar ratios of AMPAR to NMDAR-mediated components of excitatory postsynaptic currents. Following striatal dopamine depletion, indirect pathway neurons exhibited decreased levels of paired pulse facilitation, enhanced sensitivity to presynaptic stimulation and an increase in the relative contribution of NMDAR to the EPSC but no change in spontaneous synaptic activity. In sham-operated mice, neurons of the direct pathway exhibited lower firing frequency compared to the indirect pathway following current injection. However, in 6-OHDA-lesioned mice, in the direct pathway, firing threshold was reduced, spike frequency adaptation developed and the frequency of spontaneous activity was also reduced. In addition, changes in the properties of NMDAR kinetics suggest that these receptors were desensitised.

Discussion: Increased synchronicity between pre and postsynaptic neurons, as indicated by decreased paired pulse facilitation, and increased sensitivity to extracellular stimulation, combined with an increase in the contribution of NMDARs to the EPSC at cortico-striatal synapses, may contribute to the over-activity of indirect pathway neurons in the parkinsonian striatum. In contrast, a decrease in spontaneous activity, postsynaptic desensitisation to excitatory stimuli and spike frequency adaptation of cortico-striatal synapses may underlie under-activity of the direct pathway.

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Introduction

The striatum is the major input region of the basal ganglia, receiving many projections including dopaminergic afferents from the substantia nigra pars compacta (SNc), and glutamatergic afferents from the cerebral cortex (Albin et al., 1989). Striatal efferents follow two distinct pathways; the ‘indirect’ striatal output pathway projects to the lateral (external) segment of the globus pallidus (Gpi or GPe in primates, GP in rodents) and selectively expresses dopamine D2 and adenosine A2A receptors. Medium spiny neurons (MSNs) of the ‘direct’ striatal pathway project directly to the substantia nigra pars...
reticulata (SNr) and medial globus pallidus (GPm), selectively expressing dopamine D1 receptors (Besson et al., 1988; Gerfen et al., 1990; Schiiffmann et al., 1991).

In Parkinson’s disease, degeneration of nigro-striatal neurons causes loss of striatal dopamine (Kish et al., 1988). Studies using animal models that replicate this dopamine loss indicate that it results in over-activity of the indirect pathway and under-activity of the direct pathway (Mallet et al., 2006, Tseng et al., 2001; Nisenbaum et al., 1986; Ingham et al., 1998; Picconi et al., 2003; Gubellini et al., 2002; Pang et al., 2001), along with corresponding changes in activity in the output regions of each pathway (Filion and Tremblay, 1991; Hutchison et al., 1994; Mitchell et al., 1986; Pan et al., 1985; Pan and Walters, 1988). These observations form the basis of the classical model of basal ganglia pathophysiology that is proposed to be responsible for the generation of parkinsonian symptoms (Penney and Young, 1983, 1986; Albin et al.; 1989). Indeed, in vivo studies have shown that correction of these basic abnormalities by surgical or pharmacological manipulation of the globus pallidus, SNr or subthalamic nucleus, supports the idea that these changes are responsible for symptom generation (Brotchie et al., 1991, 1993; Maneu et al., 1994; Bergman et al., 1990; Klockgether and Turski, 1993; Greenamyre and O’Brien, 1991). However, the specific changes in synaptic properties that might underlie the abnormalities in activity of the indirect and direct pathways in Parkinson’s disease remain unknown.

The unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat (Ungerstedt, 1968) is the most well-characterised and reproducible rodent model of Parkinson’s disease. Recent studies have utilised a form of the 6-OHDA-lesioned rodent model in bacterial artificial chromosome (BAC) mice to identify changes in the plasticity and function of the striatal output pathways following dopamine depletion (Kreitzer and Malenka, 2007; Shen et al., 2008; Taverna et al., 2008). These studies involved 6-OHDA administration to juvenile mice (P19–P30), with the lesion developing for 2–7 days. It is not known whether the changes described in adult rodents with stable 6-OHDA-lesions also occur in juvenile rodents acutely treated with 6-OHDA. In addition, these studies do not explain how the indirect and direct pathways become over-active and under-active, respectively, in the dopamine-depleted striatum. Indeed, the changes in synaptic plasticity described would not explain how the indirect and direct pathways become under-active and over-active respectively and are suggested as being mechanisms to compensate for loss of dopamine, and thus attempt to counter the changes in pathway activity that are classically thought to underlie symptom generation. Thus, we examined presynaptic and postsynaptic properties of glutamatergic cortico-striatal synapses in a stable 6-OHDA-lesioned model in adult BAC mice and attempted to identify changes in the properties of these synapses caused by dopamine depletion. The properties we characterise in this paper include: paired pulse facilitation, spontaneous activity, the magnitude of the N-methyl-D-aspartate receptor (NMDAR) excitatory postsynaptic current (EPSC) relative to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor (AMPAR) EPSC, AMPAR and NMDAR kinetics, and the intrinsic excitability of the postsynaptic MSNs of each striatal output pathway.

Methods

Animals

BAC transgenic mice in which A2A (indirect pathway) or D1 (direct pathway) receptor expression is reported by co-expression of eGFP (A2A-eGFP, D1-eGFP) (Gong et al., 2003) were obtained from the Mutant Mouse Regional Resource Center (MMRRC). Mice were kept under a 12:12 h light–dark cycle, with free access to food and water. Anatomical data were obtained from 16 mice of both sexes (4 6-OHDA-lesioned; 4 sham-operated for each strain). Electrophysiological recordings were obtained from 26 A2A-eGFP (12 sham-operated; 14 6-OHDA-lesioned) and 20 D1-eGFP (10 sham-operated; 10 6-OHDA-lesioned) mice. All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care and the Animal Care Committees of the University of Toronto and the University Health Network.

Unilateral 6-OHDA-lesions

Thirty minutes prior to surgery, desipramine hydrochloride (25 mg/kg, Sigma Aldrich) and pargyline hydrochloride (5 mg/kg, Sigma Aldrich) (0.9% sterile saline, pH 7.4) were systemically administered (i.p.). Mice (P31–42) were anaesthetised (isoflurane (Abott), 2–3%) and placed in a stereotaxic frame (David Kopf Instruments, USA). 6-OHDA (15 μg/μl, 0.02% ascorbic acid, w/v in 0.9% saline) or vehicle (sham) was unilaterally injected into the medial forebrain bundle (MBF) (rate 0.1 μl/min, 3 μg total) at the following coordinates: AP: −1.2 mm, ML: −1.1 mm, and DV: −5.0 mm (Franklin and Paxinos, 2007; Iancu et al., 2005).

Confocal microscopy

At least 21 days post lesion, mice were anaesthetised (isoflurane 2–3%) and transcardially perfused with PBS (4 °C), followed by 4% paraformaldehyde in PBS (4 °C). Brains were removed and cryoprotected using 30% sucrose in PBS. Cryostat-cut coronal sections (20 μm) were generated and mounted on slides (Fluorescent mounting medium, Dako, USA). For each animal, 3 sections were retained for assessment of striatal dopamine depletion using tyrosine hydroxylase immunohistochemistry (see Supplementary data, Fig. S1). Images showing BAC driven eGFP expression in the striatum, GP and SNr of sections of were obtained using a LSM5 Lasermodul confocal system in combination with a LSM510 microscope, 40x oil-immersion objective, BP 505–530 filter set and 488 nm argon laser excitation (Zeiss). In order to quantify the number of eGFP fluorescent cells in the region of the striatum utilised for electrophysiological studies, the number of cells in a 230 μm² area of the rostral dorso-lateral striatum (AP: +1.7 mm from Bregma according to Franklin and Paxinos; 2007), ipsiversive to the sham-operation or 6-OHDA-lesion was counted. For sections of GP and SNr, 3 areas of 25 μm × 25 μm were chosen randomly in each section, and the fluorescence intensity was determined. Background fluorescence was accounted for by subtraction of an intensity reading taken from the cortex of each slice.

Electrophysiological recording

Fifteen days post lesion, mice underwent behavioural testing to assess striatal dopamine depletion (see Supplementary data, Fig. S2). 6-OHDA-lesioned animals that failed to display significant asymmetry in behaviour were deemed unlesioned and excluded from the study. Nineteen to 30 days post lesion, mice (P50–P71) were anaesthetised (ketamine (150 mg/kg) and xylazine (10 mg/kg), CDNM, Canada), and transcardially perfused with ACSF (4 °C) (in mM: NaCl 125, KCl 2.5, NaHCO3 25, Na2HPO4 1.25, CaCl2 2, MgCl2 1.2, Glucose 25 (Bioshop, Canada), aerated with 95% O2/5% CO2). Brains were removed, and sagittal slices (230 μm) prepared using a vibratome (Leica Microsystems, Germany) whilst submerged in aCSF (4 °C). Slices were transferred to aCSF (36 °C) for 30 min and then equilibrated for a further 30 min (25 °C) before use. A single slice from each hemisphere was retained to confirm striatal dopamine depletion by HPLC (see Supplementary data, Fig. S2). Electrophysiological and behavioural data obtained from mice with ~95% striatal dopamine depletion were excluded from the study.

MSNs were identified visually using infrared-differential interference microscopy (IR-DIC), and eGFP expression confirmed using epifluorescence (Axioskop 2 with HBO-50 illuminator, Zeiss). Patch
clamp recordings were made in aCSF containing 50 μM picrotxin (25 °C) using borosilicate glass pipettes (3–5 MΩ) (Clark, Warner Instruments, USA) filled with (in mM): KMeSO4 119, KCl 12, MgCl2 1, EGTA 1, CaCl2 0.1, HEPES 10, Na-GTP 0.4, Mg-ATP 2 and phosphocreatine 10 (Sigma Aldrich). Recordings were discarded when access resistance changed by >20%. Data were acquired, sampled at 20 kHz and filtered at 10 kHz, except for acquisition of spontaneous and miniature EPSCs (sEPSC and mEPSC, respectively), which were sampled at 25 kHz and filtered at 5 kHz (Axopatch 200B amplifier and 1322A digidata board, Molecular Devices, USA).

The intrinsic membrane properties of each cell were determined in voltage clamp by applying a 5 mV hyperpolarising step from a holding potential of −80 mV. Parameters including cell capacitance (pf), membrane resistance (in MΩ) and membrane time constant (in ms) were measured using the membrane test function of the pClamp 8 software (Axon Instruments, USA). Subsequently, the following whole cell current injection and extracellular stimulation protocols were applied. Except for sEPSCs and mEPSCs, which were analysed using Mini Analysis software (Synaptosoft, Inc. USA), all analysis was conducted in Clampfit versions 8 and 10 (Axon Instruments, USA).

Current–voltage relationship and spike frequency (current clamp): Current injections were applied for 500 ms, increasing in 50 pA steps, from −100 pA to +450 pA (3 repeated trials). Changes in membrane potential were measured at the end of each pulse, and mean spike frequency was calculated. sEPSC and mEPSC frequency and amplitude (voltage clamp): Cells were held at −80 mV and baseline spontaneous activity was recorded for 5 min. Following this, the non-selective K+ channel blocker, 4-aminopyridine (4-AP, 100 μM) (Flores-Hernández et al., 1994) was applied for 15 min and the change in activity recorded. Finally, 4-AP was exchanged for TTX (1 μM) until a consistent blockade of sEPSCs was observed and the resultant mEPSC activity was recorded for 5 min. Events were detected by setting the threshold value for detection at four times the root mean square background noise, followed by visual confirmation of sEPSP or mEPSP detection. For the construction of cumulative probability plots 200 successive events were used.

Extracellular stimulations: A bipolar stimulating electrode (FHC, USA) was placed in the anterior portion of the deep layers of the motor cortex, adjacent to the white matter that overlies the dorso-lateral striatum. The approximate distance between the stimulating and recording electrodes was 0.5 mm. MSNs were held in either voltage clamp or current clamp as described below and, unless stated, the extracellular stimulus was adjusted to produce an EPSC/P of 250–500 pA or 5–10 mV. Stimulus intensities ranged from 0.1–0.6 ms in duration and 0.05–20 mA in amplitude, with a mean duration of 0.14 ± 0.02 ms and a mean amplitude of 1.2 ± 0.5 mA (N = 48). Determination of current threshold for evoked EPSC (voltage clamp): MSNs were held at −80 mV, and a series of pulses of increasing intensity (0.55–3 mA) were applied with duration 0.1 ms. The threshold stimulus required to evoke an EPSC as well as the amplitudes of the EPSCs evoked with varying stimulations and AMPAR and NMDAR kinetics were identified using one-way ANOVA. Changes in current–voltage relationship, paired pulse ratio, sEPSCs and mEPSCs were analysed using two-way ANOVA with Bonferroni’s multiple comparisons test. For the electrophysiological studies, N indicates the number of recorded cells, and is followed by the number of mice used to obtain recordings. Significant differences in resting membrane potential (RMP), capactance, membrane resistance, membrane time constant, EPSC amplitude at varying stimulations and AMPAR and NMDAR kinetics were identified using one-way ANOVA. Changes in current–voltage relationship, paired pulse ratio, sEPSCs and mEPSCs were analysed using two-way ANOVA with Bonferroni’s multiple comparison test post-hoc, using surgical state of animal (sham-operated or 6-OHDA-lesioned) and membrane potential (mV) for current–voltage relationship experiments, inter-stimulus interval (ms) for PPR experiments and frequency (Hz) and amplitude (pA) for sEPSC/mEPSC experiments as variables. Differences in the ratio of AMPAR to NMDAR EPSCs and also in the threshold stimulation intensities required to elicit an evoked EPSC were determined using Student’s unpaired t-test. AMPAR/NMDAR are presented as box plots; bars represent maximum and minimum values and the box contains the median value bounded by the 25th and 75th percentiles.

Results

Striatal dopamine depletion has no effect on pathway specific expression of A2A or D1 receptors or axonal trajectories of striatal outputs

To ensure that any changes in activity of the striatal output pathways observed following dopamine depletion could not be attributed to loss of pathway selective expression of D1 and A2A receptors, their pattern of expression in both the striatum and terminal fields of the direct and indirect pathways, i.e. SNr and GP was assessed. Since electrophysiological recordings were performed in the rostral dorso-lateral striatum, striatal cell counts were confined to this region. In sham-operated D1-eGFP and A2A-eGFP mice, eGFP expression was observed within cell bodies of striatal MSNs. In D1-eGFP mice, the intensity of eGFP and number of eGFP-positive MSNs in the rostral striatum was significantly lower compared to A2A-eGFP mice (A2A-eGFP mice (sham): 35 ± 4 cells, N = 4; D1-eGFP mice (sham): 19 ± 9 cells, N = 4, p < 0.05). Following 6-OHDA-lesion, there was no significant difference in the number of eGFP-positive cells within the striatum in either strain compared to control (A2A-eGFP mice (6-OHDA): 34 ± 3 cells, N = 4; D1-eGFP mice (6-OHDA): 15 ± 6 cells, N = 4, p > 0.05 compared to sham-operated) (Figs. 1A,B). In sham-operated A2A-eGFP mice, significantly higher amounts of eGFP expression were observed in the GP compared to sham-operated D1-eGFP mice (GP of A2A-eGFP mice (sham): 432 ± 148 fluorescence units, N = 4; GP of D1-eGFP mice (sham): 5 ± 76 fluorescence units,
N = 4, p < 0.01), reflecting the trajectory of the indirect pathway as has previously been described (Gerfen et al., 1990). Conversely, there seemed to be a higher level of eGFP expression in the SNr of sham-operated D1-eGFP mice compared to sham-operated A2A-eGFP mice (SNr of A2A-eGFP mice (sham): 0 ± 41 fluorescence units, N = 4; SNr of D1-eGFP mice (sham): 142 ± 91 fluorescence units, N = 4), reflecting the trajectory of the direct pathway (Besson et al., 1988). However, because of wide variation in data due to the low levels of fluorescence in the D1-eGFP mouse this result was not significant. Following 6-OHDA-lesion, there was no significant difference in the pattern of eGFP expression within the GP and SNr compared to sham-operated animals (Figs. 1A,C,D). For imaging studies, the extent of striatal dopaminergic depletions was confirmed in each mouse using tyrosine hydroxylase (TH) staining (Fig. S1).

The sensitivity to glutamate release is increased at cortico-striatal synapses in A2A-eGFP MSNs of 6-OHDA-lesioned mice

A key factor in regulating the activity of striatal outputs is the degree of presynaptic neurotransmitter release at cortico-striatal synapses, which can be monitored using measurements of paired pulse facilitation (Dobrunz and Stevens, 1997; Zucker, 1973). In sham-operated mice, paired stimulations with an ISI of 25, 50 or 100 ms, initiated in voltage clamp, facilitated the second EPSC in both groups with the greatest paired stimulations with an ISI of 25, 50 or 100 ms, initiated in voltage clamp, facilitated the second EPSC in both groups with the greatest facilitation observed with an ISI of 50 ms in A2A-eGFP MSNs and with an ISI of 25 ms in D1-eGFP MSNs (paired pulse ratio (PPR) = A2A-eGFP MSNs, 25 ms ISI: 1.16 ± 0.04; 50 ms ISI: 1.26 ± 0.06; 100 ms ISI: 1.10 ± 0.04, N = 11, 7 mice, and D1-eGFP MSNs, 25 ms ISI: 1.30 ± 0.11; 50 ms ISI: 1.23 ± 0.05; 100 ms ISI: 1.20 ± 0.08, N = 8, 5 mice). There was no significant difference between the two sham-operated groups following any ISI (Figs. 2A,B,E,F). In 6-OHDA-lesioned mice, MSNs expressing A2A-eGFP showed decreases in PPR with ISIs of 25, 50 and 100 ms compared to sham-operated mice, with the decrease at 50 ms being highly significant (A2A-eGFP MSNs (6-OHDA), 25 ms ISI: 1.08 ± 0.02, p < 0.05; 50 ms ISI: 1.05 ± 0.03, p < 0.001, and 100 ms ISI: 0.98 ± 0.02, p < 0.05, N = 12, 6 mice). There was no significant difference in response to the paired pulse stimulation protocol in D1-eGFP MSNs of 6-OHDA-lesioned mice compared to sham-operated mice (Figs. 2A,B,E,F).

In order to identify how the change in PPR observed in 6-OHDA-lesioned mice affects postsynaptic neuronal integration the same protocol was carried out in current clamp. In this protocol the focus was on ISIs where facilitation was observed in sham-operated animals using voltage clamp (25, 50, and 100 ms). In sham-operated animals paired pulse facilitation was observed at all ISIs examined in A2A-eGFP MSNs (A2A-eGFP MSNs (sham), 25 ms ISI: 1.44 ± 0.22, 50 ms ISI: 1.31 ± 0.09, and 100 ms: 1.29 ± 0.18, N = 6, 4 mice). In D1-eGFP MSNs paired pulse facilitation was observed at ISIs of 50 and 100 ms but not at 25 ms (D1-eGFP MSNs (sham), 25 ms ISI: 0.99 ± 0.15, 50 ms ISI: 1.38 ± 0.29, and 100 ms ISI: 1.15 ± 0.11, N = 4, 3 mice). In 6-OHDA-lesioned mice the PPR observed in A2A-eGFP MSNs was significantly decreased at an ISI of 25 ms compared to A2A-eGFP MSNs in sham-operated mice (A2A-eGFP MSNs (6-OHDA), 25 ms ISI: 0.77 ± 0.11 p < 0.05, 50 ms ISI: 1.10 ± 0.17 and 100 ms ISI: 0.98 ± 0.15, N = 5, 3 mice). This change in synaptic function was not observed in D1-eGFP MSNs from 6-OHDA-lesioned mice (Figs. 2C,D,G,H).

Next we analysed the evoked firing threshold of cortico-striatal synapses. In sham-operated animals, there was no significant difference between the threshold stimulus intensity required to evoke EPSPs in A2A-eGFP MSNs (225 ± 25 μA, N = 4, 3 mice) compared to D1-eGFP MSNs (183 ± 34 μA, N = 8, 5 mice). In addition to the decrease in paired pulse facilitation observed in A2A-eGFP MSNs from 6-OHDA-lesioned mice compared to sham-operated, the strength of cortical stimulation required to evoke an EPSP was decreased by approximately 59% (34 ± 17 μA, N = 6, 4 mice, p < 0.01). Thus, there is a dramatic decrease in the firing threshold of cortico-striatal synapses following striatal dopamine depletion. In D1-eGFP mice, striatal dopamine depletion had no
significant effect on the stimulus intensity required to evoke EPSCs (220 ± 88 μA, N = 10, 5 mice) (Fig. 3). To determine whether there was a change in the relationship between stimulus strength and EPSC amplitude, the response to threshold, 2 times threshold and 3 times threshold stimulation was also tested in both pathways in sham-operated and 6-OHDA-lesioned groups in the two strains of animals. Neither pathway nor surgical conditions had a significant effect on the response (data not shown).

Dopamine depletion decreases spontaneous activity at cortico-striatal synapses of D1-eGFP MSNs

In MSNs of the indirect and direct pathways the frequency of mEPSCs has been reported to be low, at approximately 2.0–2.9 Hz for the indirect pathway and 1.8–2.0 Hz for the direct pathway (Day et al. 2006; Cepeda et al. 2008), reflecting low levels of spontaneous quantal release of neurotransmitters independent of action potentials. We were also interested in whether the direct and indirect pathways responded differently to action potentials that were not electrically evoked, and whether dopamine depletion affected this. Since MSNs within the striatum are usually quiescent, sEPSCs were generated by adding the K+ channel blocker, 4-AP to the recording chamber (Flores-Hernández et al., 1994), and then TTX was added to block action potential firing. In sham-operated mice, under basal conditions (no 4-AP or TTX), the frequency of sEPSCs was 2.33 ± 0.27 Hz and 1.57 ± 0.12 Hz in A2A-eGFP MSNs and D1-eGFP MSNs respectively, with no significant difference between the two pathways (both N = 3, 3

Fig. 2. Chronic dopamine depletion decreases paired pulse facilitation at cortico-striatal synapses onto A2A-eGFP MSNs. Sample traces of recordings made in voltage clamp from (A) A2A-eGFP and (B) D1-eGFP MSNs, and in current clamp from (C) A2A-eGFP and (D) D1-eGFP MSNs in sham-operated and 6-OHDA-lesioned mice. Arrows indicate the time of extracellular stimulations. Effect of striatal dopamine depletion on paired pulse ratio in A2A-eGFP MSNs: (E) recorded in voltage clamp, (G) recorded in current clamp; and D1-eGFP mice MSNs: (F) recorded in voltage clamp, (H) recorded in current clamp. Solid and open shapes represent data from sham-operated and 6-OHDA-lesioned mice respectively. (E and G) Dopamine depletion reduces the magnitude of paired pulse facilitation observed in A2A-eGFP MSNs (voltage clamp: $F_{1,126} = 15.52$, *** indicates $p < 0.001$, two-way ANOVA with Bonferroni’s multiple comparison test post-hoc; sham-operated: N = 11, 7 mice; 6-OHDA-lesioned: N = 12, 6 mice; current clamp: $F_{1,27} = 8.78$, * indicates $p < 0.05$, sham-operated: N = 6, 4 mice; 6-OHDA-lesioned: N = 5, 3 mice). (D and H) Dopamine depletion has no effect on paired pulse facilitation in D1-eGFP (voltage clamp: sham-operated: N = 8, 5 mice, 6-OHDA-lesioned: N = 8, 5 mice; current clamp: sham-operated: N = 4, 3 mice, 6-OHDA-lesioned: N = 4, 3 mice).

Fig. 3. Dopamine depletion decreases the threshold stimulation intensity required to evoke EPSCs in the indirect pathway. Bar graphs to show the mean threshold stimulation intensity (μA) ± SEM required to evoke an EPSC in MSNs from the (A) indirect and (B) direct pathways in sham-operated (grey bars) and 6-OHDA-lesioned (white bars) BAC mice. (**) indicates $p < 0.01$ compared to sham-operated mice, two-way ANOVA with Bonferroni’s multiple comparison test post-hoc; A2A-eGFP MSNs: N = 8, 5 mice; D1-eGFP MSNs: N = 8, 5 mice.)
In 6-OHDA-lesioned mice, there was no change in the frequency of basal sEPSCs in A2A-eGFP neurons, but there was a significant decrease in firing frequency in D1-eGFP neurons to 0.27±0.10 Hz (p<0.01, N=5, 4 mice) (Figs. 4A–D). The peak amplitudes of sEPSCs in each pathway were 12.87±0.83 pA for A2A-eGFP MSNs and 12.89±1.00 pA for D1-eGFP MSNs from sham-operated animals with no significant difference in 6-OHDA-lesioned animals (Figs. 4E,F).

In sham-operated mice, the frequency of sEPSCs increased to 4.83±0.23 Hz and 5.05±0.34 Hz in A2A-eGFP MSNs and D1-eGFP MSNs respectively in response to the application of 4-AP. The amplitudes of the sEPSCs were 21.33±3.00 pA and 19.94±1.72 pA for A2A-eGFP and D1-eGFP MSNs respectively. In 6-OHDA-lesioned animals the frequency and amplitude of sEPSCs in response to 4-AP was not significantly different in A2A-eGFP MSNs (Figs. 4C,E). In D1-eGFP MSNs, sEPSC frequency was significantly decreased (1.80±0.37 Hz; p<0.001) (Fig. 4D), but there was no significant change in amplitude (Fig. 4F).

Dopamine depletion differentially alters NMDAR function at cortico-striatal synapses of A2A-eGFP MSNs and D1-eGFP MSNs

Following 6-OHDA-lesion of the nigro-striatal pathway, it has been shown that striatal NMDAR function is increased; however, it is

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**Fig. 4.** Dopamine depletion decreases the frequency of spontaneous activity in D1-eGFP MSNs. (A and B) Example traces showing the spontaneous and miniature EPSC (sEPSC and mEPSC respectively) activity in A2A-eGFP MSNs (A) and D1-eGFP MSNs (B) under basal conditions, and in the presence of 4-AP (100 μM) or TTX (1 μM) in sham-operated and 6-OHDA-lesioned mice. (C and D) The frequency of eEPSCs and mEPSCs at baseline, in response to 4-AP, and in response to TTX, is not affected by dopamine depletion in A2A-eGFP MSNs (C), but is significantly reduced in all three conditions in D1-eGFP MSNs (D) (F$_{1,17}$ = 91.59. **, *** indicate p<0.01 and p<0.001 respectively, two-way ANOVA with Bonferroni’s multiple comparison test post-hoc). A2A-eGFP MSN sham-operated: N=3, 3 mice, 6-OHDA-lesioned: N=3, 3 mice, D1-eGFP MSN sham-operated: N=3, 3 mice, 6-OHDA-lesioned: N=5, 4 mice). (E and F) The amplitude of eEPSCs and mEPSCs is not affected by dopamine depletion in A2A-eGFP or D1-eGFP MSNs.
unclear whether this is specific for one of the striatal output pathways or both (Calabresi et al., 1993; Nash et al., 1999, 2000; Nash and Brodchie, 2000, 2002). Thus, the effect of 6-OHDA-lesion on the ratio of the AMPAR to NMDAR-mediated components of the excitatory current was examined at cortico-striatal synapses. These studies were performed at a holding potential of −85 mV under Mg2⁺-free conditions, ensuring no Mg2⁺-block of NMDARs. In sham-operated mice, the AMPAR:NMDAR was 2.4±0.1 ms (AMPAR:NMDAR ratio in D1-eGFP MSNs (5.61±0.77, fi p < 0.01, N = 9, 6 mice) and 5.56±1.14 in D1-eGFP MSNs (N = 5, 3 mice), with no significant difference between the two. In 6-OHDA-lesioned mice, the AMPAR:NMDAR was significantly decreased in A2A-eGFP MSNs (4.62±0.38, p < 0.01, N = 9, 6 mice) compared to sham-operated mice. However, striatal dopamine depletion had no effect on the AMPAR:NMDAR ratio in D1-eGFP MSNs (5.61±0.77, N = 6, 5 mice) (Fig. 5).

We also compared the kinetics of the AMPAR and NMDAR EPSCs in MSNs of the indirect versus direct pathway and whether these were affected by striatal dopamine depletion. In sham-operated mice, the mean 10–90% rise time of the AMPAR EPSC in A2A-eGFP and D1-eGFP MSNs was 2.4±0.1 ms (N = 8, 6 mice), and 2.5±0.3 ms (N = 5, 3 mice) respectively (Figs. 6A,B). This was unchanged by dopamine depletion (data not shown). The decay phase of the AMPAR EPSC was best fitted by a single exponential curve (Ozawa et al. 1998). In sham-operated mice, in A2A-eGFP MSNs, the time constant of the decay of the AMPAR EPSC was 6.8±0.4 ms; conversely, in D1-eGFP MSNs the decay time constant was significantly slower at 9.3±0.3 ms (N = 5, 3 mice, p < 0.05). Dopamine depletion did not change the AMPAR EPSC decay time constant in A2A-eGFP MSNs but it did significantly decrease the decay time constant in D1-eGFP MSNs to 7.7±0.3 ms (N = 6, 5 mice, p < 0.05), which is similar to the decay observed in A2A-eGFP MSNs (Figs. 6A,B).

In A2A-eGFP expressing MSNs from sham-operated mice the mean 10–90% rise time of the NMDA EPSC was 7.5±0.4 ms (N = 8, 6 mice). As previously described (Lester et al., 1990), the time constant of the decay of the NMDAR EPSC was best fitted by a biexponential curve with a 'fast' time constant (τfast) of 40.6±4.0 ms and a 'slow' time constant (τslow) of 623.8±1567.0 ms (N = 8, 6 mice). The fast component contributed 71±3% of the decay phase. In D1-eGFP MSNs from sham-operated mice the 10–90% rise time of the NMDAR EPSC was significantly slower compared to A2A-eGFP MSNs (11.5±1.8 ms (p < 0.001, N = 5, 3 mice). However, the NMDAR EPSC decay constants were similar to those of A2A-eGFP MSNs with τfast equal to 56.3±15.8 ms and τslow equal to 662.6±269.4 ms; the fast decay component contributed to 69±4% of the decay phase of the NMDAR EPSC (N = 5, 3 mice) (Figs. 6C,D,E,F).

Dopamine depletion did not significantly affect the kinetics of the NMDAR EPSC in the indirect pathway of 6-OHDA-lesioned mice (A2A-eGFP MSN (6-OHDA) NMDAR 10–90% rise time: 7.8±0.5 ms, τfast: 36.1±2.9 ms, τslow: 421.9±79.8 ms, τfast contribution: 76±3% (N = 9, 6 mice). However, in the direct pathway, in 6-OHDA-lesioned mice, there was a significant slowing of the fast phase of the NMDAR EPSC decay compared to sham-operated mice (D1-eGFP MSNs (6-OHDA) NMDAR, τfast: 113.6±30.1 ms, p < 0.01, N = 6, 5 mice). In addition the contribution of the fast component to the decay phase of the NMDAR EPSC was significantly decreased to 58±5% (p < 0.05). The 10–90% rise time and τslow of the NMDAR EPSC in D1-eGFP MSNs from 6-OHDA-lesioned mice were not significantly affected (D1-eGFP MSNs (6-OHDA) 10–90% rise time: 10.3±0.8 ms, τslow: 989.2±358.4 ms, N = 6, 5 mice) (Figs. 6C,D,E,F). Taken together, these data suggest that there is an increase in the NMDAR component of the postsynaptic response on the indirect pathway following striatal dopamine depletion, however, the properties of the NMDARs are not altered in these neurons. Conversely, in MSNs of the direct pathway, whilst the relative contribution of NMDARs to the EPSC does not change in 6-OHDA-lesioned mice compared to sham-operated animals, there is a change in the properties of AMPARs and NMDARs, such that both close more slowly.

D1-eGFP expressing MSNs exhibited altered intrinsic properties and spike frequency adaptation in 6-OHDA-lesioned mice

The intrinsic neuronal properties, resting membrane potential (RMP), cell capacitance, membrane resistance and time constant were also measured. In sham-operated mice, the RMP of A2A-eGFP MSNs was −85.0±0.7 mV, (N = 15, 8 mice), whereas in D1-eGFP MSNs it was −89.6±1.8 mV (N = 10, 6 mice, p < 0.05). The RMP of neither MSN population was affected by dopamine depletion (6-OHDA-lesioned: A2A-eGFP MSNs: −84.9±0.7 mV, N = 19, 9 mice, D1-eGFP MSNs: −89.9±0.9 mV, N = 11, 5 mice) (Fig. 7A). In A2A-eGFP MSNs from sham-operated mice, the cell capacitance was 99.8±3.2 pF (N = 15, 8 mice), which was significantly lower than in D1-eGFP MSNs, where it was 127.2±11.1 pF (p < 0.01, N = 10, 6 mice). In 6-OHDA-lesioned mice the cell capacitance of D1-eGFP MSNs was significantly reduced compared to sham-operated animals (D1-eGFP MSNs (6-OHDA): 101.5±4.3 pF, p < 0.05 compared to D1-eGFP MSNs (sham), N = 11, 5 mice) to a level comparable to that observed in
A2A-eGFP MSNs. In A2A-eGFP MSNs, striatal dopamine depletion had no significant effect on cell capacitance (A2A-eGFP MSNs (6-OHDA): 90.7 ± 3.0 pF, N = 19, 9 mice) (Fig. 7B).

In sham-operated mice the membrane resistance was the same in MSNs from both the indirect and direct pathways (A2A-eGFP MSNs (sham): 58.9 ± 4.4 MΩ, N = 15, 8 mice; D1-eGFP MSNs (sham): 79.0 ± 4.4 MΩ, N = 10, 6 mice; D1-eGFP MSNs (6-OHDA-lesioned): N = 11, 5 mice).
10.7 MΩ, N = 10, 6 mice). In contrast, in 6-OHDA-lesioned mice the membrane resistance was significantly increased in D1-eGFP MSNs but not in A2A-eGFP MSNs (A2A-eGFP MSN (6-OHDA): 66.6 ± 6.5 MΩ, N = 19, 9 mice; D1-eGFP MSN (6-OHDA): 159.2 ± 24.4 MΩ, p < 0.01 compared to D1-eGFP MSN (sham), N = 11, 5 mice) (Fig. 7C). The membrane time constant was 2.2 ± 0.1 ms (N = 15, 8 mice) in A2A-eGFP MSNs and 2.2 ± 0.1 ms (N = 10, 6 mice) in D1-eGFP MSNs from sham-operated mice. This was unchanged in 6-OHDA-lesioned animals (data not shown).

Dopamine depletion also had no effect on the current–voltage relationship or action potential firing properties of A2A-eGFP MSNs. In contrast, in D1-eGFP MSNs from 6-OHDA-lesioned mice, current injections of 100–450 pA produced membrane potential depolarisations that were significantly greater than those observed in D1-eGFP MSNs from sham-operated mice (Table 1, Fig. 8F). This is most likely due to the observed increase in membrane resistance. Following current injection of 250 pA, in D1-eGFP MSNs from sham-operated mice, the mean membrane depolarisation was 55.4 ± 2.3 mV (N = 10, 6 mice) compared to 43.2 ± 1.7 mV (N = 11, 5 mice, p < 0.001) in 6-OHDA-lesioned mice. Thus, a 250 pA current injection into D1-eGFP MSNs resulted in a subthreshold membrane potential depolarisation in sham-operated mice and action potential firing in some, but not all 6-OHDA-lesioned mice. Thus, in D1-eGFP mice when comparing the relationship between current injection and frequency of firing (Fig. 8G), overall there was no significant difference in the firing frequency when sham-operated and 6-OHDA-lesioned mice. Furthermore, when the relationship between membrane potential and firing frequency was compared, even though D1-eGFP MSNs from 6-OHDA-lesioned mice reached firing threshold with less stimulation, their ability to fire action potentials and the frequency of action potential firing was significantly decreased (Fig. 8H). As stated previously, in D1-eGFP MSNs from 6-OHDA-lesioned mice, a current injection of 250 pA depolarised the postsynaptic membrane to −43.2 mV. In D1-eGFP MSNs from sham-operated mice, a larger amount of current injection (400 pA) was required to produce a similar amount of membrane depolarisation (−43.8 ± 1.1 mV, N = 10, 6 mice). When the frequency of action potential firing at these two different current injections, with similar membrane depolarisations was compared, in D1 MSNs from sham-operated animals, injection of 400 pA of current generated an action potential firing frequency of 15.6 ± 2.6 Hz (N = 10, 6 mice) compared to an action potential frequency of 4.9 ± 1.9 Hz in D1 MSNs from 6-OHDA-lesioned mice, at a current injection of 250 pA (N = 11, 5 mice). Thus, despite there being a similar level of depolarisation in both groups, there is a higher action potential firing frequency in D1 MSNs of sham-operated animals compared to D1 MSNs of 6-OHDA-lesioned mice. Additionally, with current injections of 350 pA or greater, D1-eGFP MSNs from 6-OHDA-lesioned mice showed spike frequency adaptation, that is action potentials cease to fire before the depolarising stimulation is removed from the cell. This occurred in 7 out of 11 D1-eGFP MSNs recorded from 4 out of 5 mice (Figs. 8E,H).

**Discussion**

**Effect of 6-OHDA-lesion on the axonal trajectory of striatal outputs**

In this study, in order to characterise the morphologically identical striatal output pathways in the parkinsonian striatum in isolation, we combined two powerful models: the chronically lesioned 6-OHDA rodent model of Parkinson’s disease and BAC-eGFP mice. The chronically lesioned 6-OHDA-lesioned rodent has undoubtedly proved to be the most reliable model for understanding mechanisms underlying generation of symptoms in Parkinson’s disease, and to date, the direct and indirect striatal output pathways cannot be segregated in the primate. We demonstrate that, following loss of striatal dopamine, the number of pathway specific MSNs and the trajectories of striatal outputs were not altered.

**Increased sensitivity of indirect pathway cortico-striatal synapses in the dopamine-depleted striatum**

Within the striatum, GABAergic interneurons modulate transmission of MSNs (Kita, 1996; Czubayko and Plenz, 2002; Tunstall et al., 2002), particularly parvalbumin-positive fast spiking GABAergic interneurons within the rostral dorso-lateral striatum (Plenz and Kita, 1998; Koos and Tepper, 1999; Mallet et al., 2005), which was the region where the electrophysiological recordings for this study were obtained. Since the balance between striatal dopamine and glutamate transmission is so important for motor control, this study focussed solely on understanding how striatal dopamine depletion alters glutamatergic cortico-striatal transmission. Thus, picrotoxin was used in these studies to block GABA<sub>A</sub> receptors.

Paired pulse facilitation was observed at short ISIs in corticostriatal synapses in both the indirect and direct pathways of adult sham-operated mice, which is consistent with previous studies (Ding et al., 2008; Cepeda et al., 2008; Kreitzer and Malenka, 2007). Following striatal dopamine depletion paired pulse facilitation was lost in A2A-eGFP MSNs. One potential explanation for the loss of paired pulse facilitation in A2A-eGFP MSNs following dopamine depletion is that a larger amount of glutamate was released following a single stimulation (Dobrunz and Stevens, 1997). Indeed, in A2A-eGFP MSNs from 6-OHDA-lesioned mice, we also observed a dramatic decrease in the presynaptic stimulus intensity required to evoke an EPSC.

To determine whether changes in paired pulse facilitation and increased sensitivity to presynaptic stimulation were associated with an alteration in spontaneous activity, sEPSCs were measured in the presence of 4-AP to allow spontaneous action potential firing, and also in the presence of TTX, which blocks action potential firing. Surprisingly, in the indirect pathway there was no change in either the frequency or amplitude of sEPSCs and mEPSCs. This suggests that the probability of neurotransmitter release did not change after dopaminergic depolarisation nor did the number of AMPAR expressed on the postsynaptic terminal (Isaac et al., 1995; Liao et al., 1995). Similarly, the decrease in the frequency of sEPSCs and mEPSCs observed in the direct pathway, which suggests a decrease in neurotransmitter release probability, did not result in an increase in paired pulse facilitation as would be expected. The changes in sEPSCs and mEPSCs in response to dopamine depletion observed here are in direct contradiction to those observed by Day et al. (2006), who showed that dopamine depletion resulted in a decrease in mEPSC frequency and spine number in the indirect pathway with no change in the direct pathway. The most likely explanation for the difference in results is the mouse model of Parkinson’s disease used. For their study, Day et al. (2006) obtained dopamine depletion through five consecutive

<table>
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<td>−31.6 ± 1.5</td>
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days of reserpine treatment in mice that were 17–25 days old at the time of electrophysiological recording. In the present study, dopamine depletion was obtained using a chronic 6-OHDA-lesion in mice that were 31 days or older at the time of surgery with electrophysiological recordings being obtained at least 21 days later. Thus the models are not directly comparable and the differing results may reflect various factors such as age-related properties of striatal MSNs, compensatory mechanisms within the striatum in response to chronic dopamine depletion that do not occur following acute striatal dopamine depletion by reserpine. It may be that five days following injection of 6-OHDA there is a decrease in the frequency of mEPSCs on the indirect pathway but further changes then occurred resulting in the data obtained here. Additional work in the study by Day et al. (2006) using chronically 6-OHDA-lesioned adult rats also showed a decrease in the number of spines on indirect pathway MSNs but not direct pathway MSNs. However, this was not paired with recordings of mEPSCs so it is not known whether a decrease in spine count in chronically 6-OHDA-lesioned rats also results in a decrease in mEPSCs.

Considering the data obtained in this study, it is most likely that the observed changes in the relationship between neurotransmitter release and postsynaptic response in the indirect and direct pathways are due to postsynaptic changes that are only evident when a large amount of neurotransmitter is released in a coordinated and repetitive fashion. One explanation could be a change in AMPAR desensitisation at the postsynaptic terminal caused by alterations in postsynaptic accessory proteins. Recent work in the hippocampus has identified a 44 kDa protein termed cystine-knot AMPAR modulating protein (CKAMP44). Over-expression of the protein does not change the frequency or amplitude of mEPSCs in hippocampal neurons but by increasing receptor desensitisation and slowing recovery from desensitisation it does eliminate the paired pulse facilitation that is normally observed (Engelhardt et al., 2010). Although CKAMP44 is not expressed in the striatum, it is possible that a similar striatal

Fig. 8. Dopamine depletion increases the excitability of D1-eGFP MSNs, which also exhibit spike frequency. Sample traces from A2A-eGFP (A), and D1-eGFP (E) MSNs in sham-operated and 6-OHDA-lesioned mice. (B and F) Graphs showing the effect of striatal dopamine depletion on the current–voltage relationship in A2A-eGFP (B) and D1-eGFP (F) MSNs. (C and G) Graphs showing the effect of striatal dopamine depletion on spike frequency following current injection in A2A-eGFP (C) and D1-eGFP (G) MSNs. (D and H) Graphs showing the relationship between the membrane depolarisation in response to current injection and spike frequency in A2A-eGFP (D) and D1-eGFP MSNs (H). Solid and open shapes represent data from sham-operated and 6-OHDA-lesioned mice respectively. (B) There was no significant difference in the response to current injection or (C and D) spike frequency in A2A-eGFP MSNs from sham-operated (N = 15, 8 mice) and 6-OHDA-lesioned mice (N = 19, 9 mice). (F) Membrane depolarisation in response to current injections between 100 and 450 pA was significantly higher in D1-eGFP MSNs from 6-OHDA-lesioned mice compared to D1-eGFP MSNs from sham-operated mice, (F1,222 = 76.78, *p < 0.05, **p < 0.01 and ***p < 0.001, two-way ANOVA with Bonferroni’s multiple comparison test post-hoc). (G) Whilst there was no statistically significant difference in the spike frequency in relation to current injection in D1-eGFP MSNs from 6-OHDA-lesioned mice, the relationship between membrane potential and spike frequency (H) is shifted to the right in D1-eGFP MSNs compared to sham-operated mice. Voltage response to current injection was plotted against spike frequency (total number of spikes per second). In the dopamine-depleted striatum attenuation of spike frequency is indicated by the plateau reached by the rightmost three points on the graph. (Sham-operated: N = 10, 6 mice, D1-eGFP 6-OHDA-lesioned: N = 11, 5 mice). Data are presented as mean ± SEM.
protein is upregulated due to dopamine depletion, which causes a slowing in AMPAR recovery from desensitisation, and so is responsible for the results obtained here (assuming it was upregulated in both pathways). In the indirect pathway it would be responsible for the loss of paired pulse facilitation with no change in the mEPSC characteristics, and in the direct pathway it would be responsible for countering the increase in paired pulse facilitation observed due to the decrease in the probability of neurotransmitter release. An alternative explanation is provided by other studies in the hippocampus that have shown that fast lateral diffusion of AMPAR within the postsynaptic density speeds recovery from paired pulse depression (Choquet, 2010; Heine et al., 2008). Immobilization of AMPAR leads to an increase in the degree of paired pulse depression observed and a similar mechanism mediated by synaptic accessory proteins could be operating here.

Other possible explanations involving changes in presynaptic properties, such as alterations in the size of the releasable pool of synaptic vesicles (Saviane et al., 2002), or the recycling properties of synaptic vesicles (Sara et al., 2005) cannot be ruled out. The changes may also reflect a loss of tonic activation of G protein-coupled receptors that affect neurotransmitter release, such as presynaptic GABA_A receptors (Nisenbaum et al., 1993), group II or III metabotropic glutamate receptors (Dohovics et al., 2003) or postsynaptic adenosine A2A receptors, which have been shown to decrease GABA release (Wirkner et al., 2004).

In addition to synapse specific alterations there are at least three possible striatal network mechanisms which could account for the selective enhancement of synaptic strength on the indirect pathway relative to the direct pathway, namely, differences in cortico-striatal afferents, inter-neuronal connectivity or retrograde messengers. The first of these relies on the finding that cortico-striatal neurons with different trajectories terminate selectively on either the direct or indirect pathway (Lei et al., 2004; Mallet et al., 2006), and these neurons are differentially effected by 6-OHDA-induced dopamine depletion (Mallet et al., 2006; Ballion et al., 2008). Cortico-striatal afferents that project inside the telencephalon (intratelencephalic (IT-type)) terminate on the direct pathway, whereas the indirect pathway is innervated by cortical afferents whose main axon terminates in the pyramidal tract (PT-type), with their collaterals terminating in the striatum (Lei et al., 2004). Dopamine depletion depresses the firing of IT-type neurons, whilst having no effect in PT neurons (Mallet et al., 2006; Ballion et al., 2008). This sparing of PT projections that terminate on the indirect pathway combined with loss of presynaptic dopamine D2 receptor regulation of glutamate release (Bamford et al., 2004; Moquin and Michael, 2009; Watanabe et al., 2009) may explain the strengthening of the response of the indirect pathway to evoked stimulation. However, a more recent study by Ballion et al. (2008) concluded that IT-type and PT-type cortico-striatal neurons innervate both striatal output pathways equally with the predominant input being provided by IT-type neurons, thus differences in the type of cortical projection may not account for the selective alteration in indirect pathway response observed in this study. A further layer of complexity is added by the finding that the axonal projection patterns of IT-type neurons arising from different regions of cortical layer V varies within the striatum (Morishima and Kawaguchi, 2006). Cortico-striatal afferents originating from deep within layer V project to the so called ‘patch’ compartment of the striatum which then innervates dopaminergic areas of the SNc, whereas cortico-striatal neurons from more superficial regions of layer V project to the so called ‘matrix’ which projects to GABAergic neurons within the SNr (Gerfen, 1992). This raises the possibility that the imbalance in pathway response could arise from location specific changes in cortico-striatal innervation and concurrent network effects.

An additional explanation for why dopamine depletion decreases PPR in the indirect pathway could relate to control by interneurons. For instance, there is evidence that the potent feed-forward inhibition provided by parvalbumin-positive fast spiking interneurons (FSI) which resides equally over both populations of striatal efferents in the lateral striatum (Mallet et al., 2005) is lost in MSNs of the indirect pathway following dopamine depletion (Mallet et al., 2006, however see Salin et al., 2009). However, this is unlikely to explain the current findings, as GABAergic transmission was blocked. It is however, possible that chronic loss of GABAergic inhibition in vivo in the 6-OHDA mice causes long-term changes in cortico-striatal synapses of the indirect pathway, which may underlie the increased sensitivity to evoked stimulation. Finally, in 6-OHDA-lesioned rats, the regulation of cholinergic interneurons over presynaptic cortico-striatal synapses is increased and decreased on the indirect and direct output pathways respectively, which may also account for the changes in pathway sensitivity observed in the present study (Salin et al., 2009).

A further explanation for the differences in the sensitivity of the direct and indirect pathways could be that the indirect pathway receives a larger proportion of action potential-dependent inputs compared to the direct pathway (Cepeda et al., 2008; Andre et al. 2010). Andre et al. (2010) also show that postsynaptic activation of D1 and D2 receptors differentially modulates glutamate release via the retrograde transport of endogenous endocannabinoids which activates presynaptic CB1 receptors. Activation of postsynaptic D2 receptors on the indirect pathway increases the release of endocannabinoids from MSNs, which results in a depression of presynaptic glutamate release due to activation of presynaptic CB1 receptors. Conversely D1 receptor activation on the direct pathway decreases the release and retrograde transport of endocannabinoids, thus decreasing the level of presynaptic CB1 receptor stimulation, resulting in an increase in the level of presynaptic glutamate release. Thus, the decrease in PPR and increased sensitivity to cortical stimulation on the indirect pathway observed here following dopamine depletion may be due to the removal of D2-endocannabinoid mediated inhibition of glutamate release. If this hypothesis was correct, then it may also be expected that the loss of cannabinoid release following dopamine depletion would also decrease glutamate release on the direct pathway. It is possible that such an effect is masked by loss of the autoinhibitory effects of presynaptic D2 receptors on cortico-striatal receptors of the direct pathway (Bamford et al., 2004; Moquin and Michael, 2009; Watanabe et al., 2009). Furthermore, previous studies have shown that endocannabinoids have a larger effect on neurotransmission in the indirect pathway compared to the direct pathway (Kreitzer and Malenka, 2007; Shen et al., 2008).

Changes in the properties of AMPARs and NMDARs at cortico-striatal synapses in the dopamine-depleted striatum

Previous studies have shown that dopamine depletion alters striatal AMPAR subunit composition (Betarbet et al. 2000; Lai et al. 2003) and phosphorylation (Ba et al. 2006) but not overall AMPAR expression (Silverdale et al. 2002). In addition NMDAR distribution (Hallett et al., 2005; Hurley et al., 2005; Gardoni et al., 2006) and function (Calabresi et al., 1993; Nash et al., 1999, 2000; Nash and Brothie, 2000; Nash and Brothie, 2002) are affected, with the changes in function being heterogenous between the direct and indirect striatal output pathways in vivo (Fantin et al., 2008). Here we confirm these observations at the synaptic level, showing that dopamine depletion has a differential effect on the two output pathways at cortico-striatal synapses. In the indirect pathway the relative contribution of NMDAR to the EPSC compared to AMPARs was increased in 6-OHDA-lesioned mice. Whilst it was not possible to determine whether this was due to a change in the amplitude of the AMPAR or NMDAR component of the EPSC in this study, changes in AMPAR expression are unlikely since the level of spontaneous activity was not altered in the indirect pathway. The increase in the relative NMDAR component is most likely to be due to increased NMDAR expression (Weihmuller et al., 1992; Hallett et al., 2005), as altered...
NMDAR subunit composition, (Nash et al., 1999; Nash and Broatchie, 2002) or NMDAR channel phosphorylation (Dunah et al., 2000) would alter the kinetics of the NMDAR response (Monyer et al., 1994), which did not occur in the indirect pathway. This change would not have been observed when spontaneous activity was measured, since the cells were held at −60 mV. Functionally, a larger NMDAR component in cortico-striatal postsynaptic excitation will lead to prolonged depolarisation of the postsynaptic membrane and increase Ca2+ entry into indirect pathway MSNs. Indeed, previous studies in 6-OHDA-lesioned rats have suggested that transmission via GluN2A-containing NMDARs is enhanced on the indirect striatal output pathway (Fantin et al., 2008).

In 6-OHDA-lesioned mice, whilst the ratio of NMDAR to AMPAR contribution was not altered in the direct pathway, the kinetics of both AMPAR and NMDARs were different. In the dopamine-intact striatum, AMPAR rise times were similar on both pathways, but the decay time was significantly slower in the direct pathway. However, following dopamine depletion there was a significant decrease in the AMPAR decay time of the direct pathway. GluA1, GluA2 and GluA3 AMPAR subunits are expressed within the striatum (Bernard et al., 1997). The slower decay time in the direct pathway of sham-operated mice may be due to differences in AMPAR subunit composition of the two pathways. The faster decay time in the direct pathway upon dopamine depletion may be a reflection of decreased GluA1 subunit expression, which has previously been reported (Lai et al., 2003; Ba et al., 2006, however see Betarbet et al., 2000) and would decrease the open time of the AMPAR channel, and may contribute to the decrease in spontaneous activity observed in the direct pathway in the parkinsonian striatum.

NMDARs on the direct pathway had a slower rise time compared to the indirect pathway whilst the decay time on both pathways was the same. NMDARs are comprised of two GluN1 and two GluN2 subunits, with the GluN2 subunit governing the pharmacology and kinetics (Ozawa et al., 1998; Monyer et al., 1994). Thus, the difference in kinetics of NMDARs between the two pathways suggests a change in the NR2 subunit composition. NMDAR-containing GluN2A subunits are preferentially expressed on the indirect pathway, whereas NMDAR-containing GluN2B subunits are preferentially expressed on the direct pathway (Fantin et al., 2007). In 6-OHDA-lesioned animals, the fast component of the decay time was dramatically increased in the direct pathway, which may reflect insertion of GluN2D subunits in the NMDAR complex, as this causes extended open times similar to those observed here (Monyer et al., 1994), however, it is currently unknown whether such an alteration occurs. Whilst NMDARs were open for longer, previous studies have shown that this causes the channel to spend a large proportion of time in a closed or desensitised state (Lester et al., 1990), meaning they would be less active, which may contribute to under-activity of the direct pathway in the parkinsonian striatum.

Changes in excitability of the direct pathway in the dopamine-depleted striatum

As has been previously reported (Gertler et al., 2008), the RMP of the indirect pathway was significantly higher compared to the direct pathway in striatal slices from adult healthy mice. In sham-operated mice, the indirect pathway was more sensitive to current injection, exhibiting a higher frequency of firing compared to the direct pathway. As previously described (Gertler et al., 2008), one of the major factors causing this difference in excitability between the two populations of striatal efferents is likely to be the relatively large surface area of neurons of the direct pathway compared to the indirect pathway, due to an increase in the number of primary dendrites. This was confirmed in our study by the larger mean cell capacitance in the direct pathway compared to the indirect pathway. Another possible explanation for the difference in excitability at rest is the lower level of synchronisation between the pre and postsynaptic terminals of cortico-striatal synapses in the direct pathway compared to the indirect pathway (Berretta et al., 1997).

Whilst the firing rate of indirect pathway was not altered following striatal dopamine depletion, the direct pathway became more sensitive to current injection, exhibiting more depolarised membrane potentials in 6-OHDA-lesioned animals compared to sham-operated animals. As suggested by the significant decrease in cell capacitance and increase in membrane resistance in this population of cells, the most likely explanation for this increase in excitability is a decrease in neuronal volume, and a change in the population of voltage-gated ion channels, respectively. The decrease in cell capacitance in the direct pathway is an interesting observation in light of previous studies which have shown that dopamine depletion results in dendritic pruning in the indirect pathway rather than the direct pathway (Day et al., 2006). Increased membrane resistance in the direct pathway is likely due to changes in the number of open potassium channels (Reyes et al. 1998), such as Kv1.2, which play a role in regulating the subthreshold excitability of in the direct pathway (Shen et al. 2004, 2007). Interestingly, despite the ability of neurons of the direct pathway to reach threshold more easily, they exhibited a lower net firing frequency, and high levels of current injection resulted in spike frequency adaptation, with action potential firing ceasing during the period of current injection. One possible mechanism for the appearance of spike frequency adaptation is an alteration in expression of other K+ channels, such as KCNQ and SK, which mediate the repolarisation and after-hyperpolarisation currents in striatal neurons and determine their ability to undergo prolonged burst firing (Ji et al., 2009; Pineda et al., 1992; Shen et al., 2005).

Thus, whilst there is an increase in intrinsic excitability in the dopamine-depleted striatum in the direct pathway, as shown by the I/V curves, since the F/mV curves show that the frequency of firing is significantly decreased, this could be one of the mechanisms responsible for under-activity of this pathway in parkinsonism. This is compounded by prolonged desensitisation of NMDARs in the direct pathway and decreased frequency of spontaneous events.

Summary and conclusion

These studies identify various abnormalities in pre and postsynaptic function of cortico-striatal synapses of the striatal output pathways of the dopamine-depleted striatum, which may be correlates of the changes in activity observed in parkinsonism. We propose that enhanced sensitivity to glutamate and an increase in the ratio of NMDA to AMPAR ratio contribute to over-activity of the indirect striatal output pathway, and that spike frequency adaptation combined with desensitisation of NMDARs and decreased spontaneous activity contributes to under-activity of the direct pathway.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.nbd.2010.10.013.
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