Inhibition of Striatal Soluble Guanylyl Cyclase-cGMP Signaling Reverses Basal Ganglia Dysfunction and Akinesia in Experimental Parkinsonism

Kuei Y. Tseng¹, Adriana Caballero¹, Alexander Dec², Daryn K. Cass¹, Natalie Simak¹, Elizabeth Sunu², Michael J. Park², Shannon R. Blume¹, Stephen Sammut²a, Diana J. Park²b, Anthony R. West²a

¹Department of Cellular and Molecular Pharmacology, Rosalind Franklin University/The Chicago Medical School, North Chicago, Illinois, United States of America
²Department of Neuroscience, Rosalind Franklin University/The Chicago Medical School, North Chicago, Illinois, United States of America

Abstract

Objective: There is clearly a necessity to identify novel non-dopaminergic mechanisms as new therapeutic targets for Parkinson’s disease (PD). Among these, the soluble guanylyl cyclase (sGC)-cGMP signaling cascade is emerging as a promising candidate for second messenger-based therapies for the amelioration of PD symptoms. In the present study, we examined the utility of the selective sGC inhibitor 1H-[1,2,4] oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) for reversing basal ganglia dysfunction and akinesia in animal models of PD.

Methods: The utility of the selective sGC inhibitor ODQ for reversing biochemical, electrophysiological, histochemical, and behavioral correlates of experimental PD was performed in 6-OHDA-lesioned rats and mice chronically treated with MPTP.

Results: We found that one systemic administration of ODQ is sufficient to reverse the characteristic elevations in striatal cGMP levels, striatal output neuron activity, and metabolic activity in the subthalamic nucleus observed in 6-OHDA-lesioned rats. The latter outcome was reproduced after intrastriatal infusion of ODQ. Systemic administration of ODQ was also effective in improving deficits in forelimb akinesia induced by 6-OHDA and MPTP.

Interpretation: Pharmacological inhibition of the sGC-cGMP signaling pathway is a promising non-dopaminergic treatment strategy for restoring basal ganglia dysfunction and attenuating motor symptoms associated with PD.

Introduction

Currently available pharmacotherapies for PD such as levodopa (L-DOPA) subdue motor symptoms via activation of striatal dopamine (DA) transmission. However, repeated L-DOPA treatment can cause severe side effects (e.g. dyskinesias), most likely as a result of abnormal changes in DA receptor expression and function [1]. Thus, there is clearly a necessity to identify novel non-dopaminergic mechanisms as new therapeutic targets for PD. Among these, the soluble guanylyl cyclase (sGC)-cGMP signaling pathway is emerging as a promising target candidate for treatment strategies aimed at restoring striatal dysfunction induced by DA cell loss [2].

It is now well accepted that sGC is the primary receptor for the gaseous neuromodulator nitric oxide [3]. Interestingly, sGC expression and activity are reportedly higher in the striatum than in any other brain region [4,5]. At the cellular level, the sGC-cGMP-PKG system is predominantly localized to striatal medium-sized spiny projection neurons (MSNs) of both the direct and indirect output pathways [5,6,7]. Until recently, the physiological function of sGC-cGMP signaling in the striatum was unclear. However, numerous recent studies now indicate that sGC-cGMP signaling is likely to function as an important cellular intermediary for regulating interactions between DA and glutamate neurotransmission in the normal and parkinsonian striatum [8,9,10,11,12,13]. In fact, findings from studies of animal models of PD indicate that following DA depletion, alterations in striatal cGMP homeostasis are likely to contribute to pathophysiological changes in basal ganglia circuits observed in PD [2]. Specifically, an upregulation of striatal sGC expression and activity (i.e., cGMP synthesis) has been observed in MPTP-treated mice [14,15]. Interestingly, transient elevations in intracellular cGMP markedly

Citation: Tseng KY, Caballero A, Dec A, Cass DK, Simak N, et al. (2011) Inhibition of Striatal Soluble Guanylyl Cyclase-cGMP Signaling Reverses Basal Ganglia Dysfunction and Akinesia in Experimental Parkinsonism. PLoS ONE 6(11): e27187. doi:10.1371/journal.pone.0027187

Editor: Mark R. Cookson, National Institutes of Health, United States of America

Received June 23, 2011; Accepted October 11, 2011; Published November 2, 2011

Copyright: © 2011 Tseng et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This research was supported by the Parkinson’s Disease Foundation and United States Public Health grants NS047452 and NS047452-S1 to ARW, and DA004093. Rosalind Franklin University start-up funds, and PRI innovation grant to KYT. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: kuei-yuan.tseng@rosalindfranklin.edu (KYT); Anthony.West@rosalindfranklin.edu (ARW)

a These authors contributed equally to this work.

b Current address: Department of Psychology, Franciscan University of Steubenville, Steubenville, Ohio, United States of America

‡ Current address: Biology Department, North Park University, Chicago, Illinois, United States of America

PLOS ONE | www.plosone.org 1 November 2011 | Volume 6 | Issue 11 | e27187
increase striatal neuronal excitability and facilitate excitatory corticostriatal synaptic transmission [12,16,17], an effect resembling that observed following chronic DA cell loss [10]. Therefore, we hypothesized that downregulation of the sGC-cGMP signaling pathway should restore pathological changes observed in the basal ganglia after chronic DA depletion, and consequently, reverse motor impairments associated with PD. Towards this goal, we examined the utility of the selective sGC inhibitor 1H-[1,2,4] oxadiazolo-[4,3-a]quinazolin-1-one (ODQ) [19,20] in reversing biochemical, electrophysiological, histochemical, and behavioral correlates of experimental PD observed in 6-OHDA-lesioned rats and mice chronically treated with MPTP.

Results

We first examined the impact of tonic cGMP signaling on corticostriatal synaptic transmission in vivo in naïve rats by measuring changes in cortically-evoked striatal local field potential (LFP) following systemic administration of the selective sGC inhibitor ODQ (Fig 1a,b). Consistent with previous studies [12], systemic administration of ODQ reduced the strength of corticostriatal transmission in a dose dependent manner (Fig 1b). Specifically, a clear attenuation of the corticostriatal postsynaptic potential (PSP) was observed 20 min after administration of 20 mg/kg, but not 10 mg/kg dose of ODQ (Fig 1b–c). This inhibition of the cortically-evoked PSP was completely restored by local (intrastriatal) infusion of the cGMP analog 8-bromoguanosine 3’5’-cyclic monophosphate sodium salt (8-Br-cGMP) (Fig 1c–d). Thus, as shown in previous studies [17], the ODQ-induced attenuation of corticostriatal synaptic transmission is mediated by local inhibition of striatal sGC and cGMP signaling. The remaining studies described below focused on assessing the utility of ODQ for reversing abnormal striatal neuronal firing activity, basal ganglia dysfunction, and akinesia observed in animal models of PD.

All 6-OHDA-lesioned rats included in the present study exhibited a marked reduction in adjusting steps executed by the contralateral forelimb (Fig 2a), and >90% depletion of DA cells in the substantia nigra (SN), as revealed by TH immunostaining (Fig 2b). We also determined the impact of 6-OHDA-induced DA depletion on striatal sGC-signaling by assessing changes in tissue cGMP levels. We found that chronic DA depletion significantly increased striatal tissue levels of cGMP by ~55%, an effect that was reversed by one systemic administration of ODQ (20 mg/Kg, i.p., Fig 2c). We next examined whether systemic administration of ODQ also reverses the abnormal striatal electrophysiological activity observed after chronic DA depletions. Consistent with previous studies [18,21], striatal single-units recorded in neurons from 6-OHDA-lesioned rats exhibited increased spontaneous firing (1.69±0.28 Hz) compared to neurons recorded in sham-operated controls (0.07±0.01 Hz) (Fig 2d–e). We found that one systemic administration of the selective sGC inhibitor ODQ (20 mg/kg, i.p.) robustly decreased the spontaneous firing observed in the DA-depleted striatum (Fig 1f). Specifically, a significant decrease in striatal firing rate from 1.72±0.49 to 0.67±0.43 Hz was observed 10 min after ODQ administration. Vehicle injection did not affect similar measures taken in 6-OHDA-lesioned rats (Fig 2f). Furthermore, ODQ administration did not affect the firing rate of striatal neurons recorded in sham-operated controls (data not shown). Together with the biochemical data, these results indicate that an upregulation of sGC signaling may contribute to the abnormal elevation of neuronal excitability observed in the DA-depleted striatum.

STN hyperactivity is another hallmark of parkinsonism as abnormal electrophysiological and metabolic changes have been consistently reported across different animal models of PD and in studies of PD patients [22]. L-DOPA treatment also normalizes STN hyperactivity [23] and mimics the therapeutic effects of STN inactivation/lesion on motor deficits observed in both PD and experimental parkinsonism [24]. We therefore evaluated the effectiveness of systemic administration of ODQ to reverse the STN hyperactivity resulting from chronic DA depletion in 6-OHDA-lesioned rats (Fig 3). Changes in the metabolic activity of the STN were assessed in sham-operated and 6-OHDA-lesioned rats by means of histochemical staining of cytochrome oxidase (CO-I) activity. All measures were taken by an investigator blind to the experimental condition. As expected, vehicle-treated 6-OHDA-lesioned rats exhibited a significant increase in STN CO-I staining when compared to the vehicle-treated sham-operated control group (Fig 3a–b). Following one systemic administration of ODQ (20 mg/kg, i.p.), the increase in STN metabolic activity was no longer detected in 6-OHDA-lesioned rats when compared to control rats. ODQ administration also significantly reduced the metabolic activity of the substantia nigra (SN), as revealed by TH immunostaining (Fig 3c). Together with the behavioral data, these results indicate that normalization of STN hyperactivity and metabolic activity of the SN following ODQ treatment are observed in this model of experimental parkinsonism.
rats (Fig 3a). To further determine whether this reversal effect of ODQ was mediated via inhibition of striatopallidal output (see Fig 2f), another cohort of sham and 6-OHDA-lesioned rats was generated to examine the effect of intrastriatal ODQ administration on STN metabolic activity. Histological examination of cannula tracks revealed that all intrastriatal microinjections were within the dorsal striatum, between 0.7 to \(0.3\) mm from bregma (see Methods for details). We found that intrastriatal infusion of ODQ (50 \(\mu\)M) was sufficient to normalize the increase of STN CO-I staining observed in the 6-OHDA group (Fig 3b).

Collectively, these observations point to the involvement of the indirect striatopallidal MSNs, as opposed to the direct striatonigral MSNs, in mediating the ODQ-dependent reversal of STN hyperactivity.

We next determined the behavioral significance of ODQ administration by measuring changes in forelimb akinesia in 6-OHDA-lesioned rats. Forelimb akinesia can be quantified in animal models of PD by means of the stepping test, which assesses behavioral parameters thought to resemble limb akinesia and gait problems seen in PD patients [25,26,27]. All 6-OHDA-lesioned rats included in this study exhibited stepping deficits (Fig 4a–c). We found that the same acute ODQ treatment used in the above biochemical, electrophysiological, and histochemical studies was also effective in reducing forelimb stepping deficits observed in 6-OHDA-lesioned rats. Moreover, the anti-akinetic effects of ODQ were found to be dose-dependent in nature, as revealed by the magnitude and duration of stepping improvement observed with increasing doses of ODQ (Fig 4d). Next, we assessed the effect of ODQ administration on stepping deficits induced by DA depletion in the chronic MPTP mouse model of PD. The MPTP model was chosen because systemic administration of this toxin induces a bilateral DA lesion that reproduces the human
We have uncovered an important role of the sGC-cGMP signaling pathway in the regulation of normal corticostriatal transmission and basal ganglia dysfunction induced by chronic DA depletion. We found that systemic administration of the selective sGC inhibitor ODQ decreased corticostriatal transmission in naïve rats in a manner that was reversed by intrastriatal infusion of a cGMP analogue. Moreover, ODQ administration markedly reversed the abnormal elevation in cGMP levels and reduced the increase in spontaneous firing observed in the DA-depleted striatum. These effects of ODQ were also associated with a normalization of the characteristic increase in metabolic activity observed in the STN following nigrostriatal DA cell loss. Moreover, the effects of systemically delivered ODQ on STN hyperactivity were replicated in studies using intrastriatal micro-injections of this drug, indicating that striatal sGC inhibition leads to downregulation of striatopallidal output. The effects of ODQ on neuronal hyperactivity in the striatum and the STN were found to be behaviorally relevant as a similar systemic treatment transiently attenuated the reduction in forelimb use observed in 6-OHDA-lesioned rats and mice chronically treated with MPTP. These observations, along with previous studies [14,15], provide strong evidence that an upregulation of sGC-cGMP signaling in the DA-depleted striatum may contribute to the enduring changes in neuronal excitability and locomotor activity observed in parkinsonian animals. Furthermore, our data demonstrate for the first time that pharmacological attenuation of striatal sGC-cGMP signaling represents a promising novel non-dopaminergic therapeutic approach for restoring basal ganglia dysfunction and subduing motor symptoms associated with PD.

Presently, little is known as to how attenuation of striatal sGC-cGMP signaling may rescue dysfunctional basal ganglia output and behavioral abnormalities associated with experimental parkinsonism. However, converging evidence now indicates that striatal sGC-cGMP signaling plays a key role in the regulation of MSN excitability [12,17], short and long-term corticostriatal synaptic plasticity [11,12,30,31,32], and neuronal synchrony [12,33,34,35]. The current studies examining the effects of ODQ on cortically-evoked striatal synaptic potentials in naïve rats indicate that tonic cGMP signaling also facilitates corticostriatal transmission within striatal networks. Taken together with previous studies [12,16,17], these findings show that transient elevations in intracellular cGMP markedly increase striatal MSN excitability and facilitate corticostriatal excitatory synaptic transmission. Notably, acute D2 (but not D1) receptor blockade mimics the facilitatory effect of DA depletion on striatal sGC activity [36,37] and MSN activity [38]. Thus, despite that striatal MSNs from both the direct and indirect output pathways express high levels of all components of the sGC-cGMP second messenger cascade [6,7], the above studies suggest that alterations in cGMP signaling observed after striatal DA-depletion could result from a preferential upregulation of cGMP synthesis in D2 receptor-expressing striatopallidal neurons of the indirect pathway [16].

Importantly, intrastriatal infusion of ODQ was sufficient to normalize the increased metabolic activity observed in the STN of 6-OHDA-lesioned rats. This finding is of great translational value as STN hyperactivity is one of the pathophysiological hallmarks of parkinsonism that has been repeatedly reported in animal models and in PD. In addition to the indirect pathway (i.e., striatopallidal neurons), there are other afferents known to contribute to the STN hyperactivity such as the excitatory inputs from the parafascicular nucleus of the thalamus and the pedunculopontine nucleus in the brainstem. However, the above outcomes from studies employing intrastriatal ODQ infusions point to a primary role of the indirect pathway in mediating both the STN hyperactivity and the ODQ-dependent reversal of this pathophysiological state induced following DA depletion.

At the cellular level, even less is known regarding how alterations in striatal sGC-cGMP signaling contribute to dysregulation of corticostriatal-striatopallidal transmission observed in the DA-depleted striatum. Given the current findings and previous reports [12,17], it is possible that inhibition of the sGC-cGMP
signaling pathway reduces the abnormal increase in intrinsic excitability observed in striatal MSNs following chronic DA depletion. Indeed, under DA-depleted conditions pharmacological downregulation of sGC-cGMP signaling (i.e., following ODQ administration) may preferentially affect striatopallidal neurons because they are likely to exhibit increased cyclic nucleotide production and PKA/PKG/DARPP-32 activation as a result of decreased D2 receptor-mediated suppression of adenylate cyclase and sGC activity [10]. This prediction is consistent with previous studies showing that drugs that augment cAMP (i.e., the adenylate cyclase activator forskolin) or cGMP (i.e., the phosphodiesterase inhibitor zaprinast) levels in MSNs increase the excitatory impact of corticostriatal transmission on these cells [17,39]. Activation of the sGC-cGMP signaling pathway is also known to stimulate presynaptic facilitation of glutamate release [40], and to increase surface expression of AMPA receptors at postsynaptic sites [41]. Taken together, these observations indicate that concurrent downregulation of pre- and postsynaptic sGC-cGMP signaling at corticostriatal synapses may be sufficient to normalize the abnormally augmented corticostriatal-striatopallidal transmission observed following DA depletion.

Unveiling the role of non-dopaminergic neural systems in the pathophysiology of experimental parkinsonism has great translational value, as this will open new avenues for treating PD and other debilitating neurological disorders. For instance, given the results of the current study, it is very likely that drugs designed to stimulate metabolism of excessive cGMP (and possibly cAMP) via activation of one or more of the numerous isoforms of phosphodiesterases expressed in the striatum [42,43] will maximize the specificity of this novel treatment approach. In support of this, in the current study we demonstrated that a second messenger-based therapy (i.e., sGC inhibition and decreased cGMP signaling) is effective for reversing basal ganglia dysfunction and akinesia induced following DA depletion.

These observations should lead to a broader understanding of how cyclic nucleotide signaling cascades can be modulated as an approach for treating motor symptoms associated with PD and related neurological disorders. Future studies will have to determine whether an enduring reversal of parkinsonian symptoms can be achieved with a treatment regimen designed to chronically downregulate striatal sGC-cGMP signaling. Moreover, novel studies examining the potential utility of combination therapy using low doses of L-DOPA and inhibitors of sGC-cGMP-PKG signaling are also warranted.

### Materials and Methods

All experimental procedures met the NIH guidelines for the care and use of laboratory animals and were approved by the Rosalind Franklin University of Medicine and Science Institutional Animal Care and Use Committee (Animal Welfare Assurance Number A3279-01, protocols 08-01 and 10–19). All animals were kept under conditions of constant temperature (21–23°C) and maintained on a 12:12 hour light/dark cycle with food and water available ad libitum. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) whereas ODQ was purchased from Tocris Bioscience (Ellisville, MO).
examined the effects of local striatal microinfusions of vehicle or the cGMP analog 8-Br-cGMP on LFPs evoked in the striatum during cortical stimulation. To this end, a concentric bipolar electrode (SNE-100; Better Hospital Equipment, Rockville Centre, NY) was used to stimulate the frontal cortex (B: 2.7 to 3.2 mm, L: 1.5 to 2.2 mm, V: 1.5 mm), while a second concentric bipolar electrode attached to a 28-gauge stainless steel cannula (Plastics One Inc., Reannex, VI) was placed in the dorsal striatum (B: ±0.5, L: 3 mm, V: ±4.3 mm) to enable the concurrent recording of evoked postsynaptic potentials (PSP) and local microinfusion of vehicle/cGMP analog (Fig 1a). Only naive adult male Sprague-Dawley (Harlan, Indianapolis, IN) rats (250–350 g) were included in this set of recordings. As previously described [11,12,16], animals were deeply anesthetized with urethane (1.5 g/kg, i.p.), placed in a stereotaxic apparatus (ASI instruments, MI) and maintained at 37°C with a heating pad (VI-20F, Fintronics Inc, Orange, CA). Cortically-evoked striatal PSPs were amplified (NeuroData, Delaware Water Gap, PA), filtered (bandwidth 0.1–100 Hz), digitized (Digilab 1442, Molecular Devices), acquired (Axoscope, Molecular Devices), and analyzed (Clampfit 10, Molecular Devices) at a sampling rate of 10 kHz. The intensity of stimulation (0.5 to 0.9 mA range of single square pulses of 0.3 ms duration delivered every 10 s) was chosen from the minimum amount of current to elicit a PSP with <15% variability in amplitude and slope. The isolated response was therefore monitored for at least 5 min to ensure the stability of the PSP, followed by 15 min of baseline PSP recording. Intrastriatal infusion of the cGMP analog 8-Br-cGMP or vehicle (aCSF) was delivered at the rate of 0.1 μl/min for 10 min using a syringe minipump (BASi Baby Bee Syringe Drives, CA).

### 6-OHDA lesion and stepping test

Adult male Sprague-Dawley (Harlan, Indianapolis, IN) rats weighing ~250 g were randomly assigned to groups receiving either 6-OHDA or vehicle (sham group). All rats were administered desipramine (10 mg/kg, i.p.) 30 min prior to surgery. Rats were then anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and placed into a stereotaxic apparatus. Eight micrograms of 6-OHDA-free base in 4 μl of 0.1% ascorbic acid was injected unilaterally into the medial forebrain bundle (B: −4.3 mm, L: +1.6 mm, V: −8.3 mm from cortical surface) [10,27]. The sham lesion was performed by injecting 4 μl of 0.1% ascorbic acid. The injection rate was 0.4 μl/min and the cannula was left in place for an additional 5 min before slowly being removed. Four weeks after surgery, the effect of 6-OHDA on forelimb akinesia was evaluated, as previously described [26,27]. All biochemical, electrophysiological, histochemical, and behavioral studies were performed >4 weeks after the 6-OHDA or vehicle infusion (average: 46±5 days).

### Quantification of striatal cGMP levels

Rats were decapitated and their brains rapidly excised on an ice-cold surface. Sections containing the striatum were collected at −80°C until further processing. The Direct cGMP ELISA Kit-Non-acylated Version (NewEast; Malvern, PA) was used to determine the levels of cGMP according to manufacturer’s instructions. For each sample, the concentration of cGMP was normalized to its protein content determined with Bio-Rad’s DC Protein Assay (Bio-Rad; Hercules, CA). For each animal, a single value was obtained per striatal sample.

---

**Figure 5. Systemic ODQ administration improves stepping performance in chronic MPTP-treated mice.** (a) Chronic MPTP treatment significantly reduced the number of TH positive neurons in the SN. The data were collected at four anatomical levels from vehicle or MPTP-treated mice 3 weeks following the last injection (**P<0.005 compared to vehicle**). (b) Images of TH immunostaining showing the degree of reduction of TH positive cells in the SN at four anatomical levels (mm from bregma): −3.1 (i, vi), −3.3 (ii, vii), −3.5 (iii, vii), and −3.7 (iv, viii). (c) Summary graph depicting the effect of chronic saline (n = 9) or MPTP (20 mg/kg, s.c., n = 13) injections on forelimb stepping performance. A significant decrease in the number of adjusting steps was observed after 6 weeks of MPTP injection (**P<0.005 vs. vehicle, Tukey post-hoc test after significant ANOVA). (d) Time course showing the effect of a single s.c. injection of vehicle and ODQ (5 and 10 mg/kg) on MPTP-induced stepping deficits (n = 5–7 mice per group). A transient improvement in stepping performance was detected 30–90 min after ODQ administration (**P<0.005, compared to measures taken at time 0 or identical time points in vehicle, Tukey post-hoc test after significant two-way ANOVA).

doi:10.1371/journal.pone.0027187.g005

**Drug preparation**

In studies involving systemic drug administration, ODQ (10–40 mg/kg) was dissolved in vehicle consisting of 10% Cremophor EL in 0.9% saline [12]. In studies using striatal ODQ microinjections, ODQ was dissolved in 0.5% DMSO in artificial cerebral spinal fluid (aCSF) and infused into the striatum (see below) at a concentration of 0.05 nmol/μL. The cGMP analog 8-Br-cGMP was dissolved in aCSF [17] and infused into the striatum (0.1 μl/min for 10 min) at a concentration of 20 nmol/μL as previously described [43–46].

**Local field potential recordings of cortical-evoked striatal postsynaptic potentials in vivo**

To determine the specificity of the pharmacological effects of the sGC inhibitor ODQ for downregulation of cGMP and the impact of this attenuation on corticostriatal transmission, we...
Single unit recordings of striatal activity in vivo

All in vivo electrophysiological recordings were conducted following the same experimental procedure as previously described [11,12,16]. Briefly, animals were deeply anesthetized with urethane (1.5 g/kg, i.p.), placed in a stereotaxic apparatus (Narishige International USA Inc) and maintained at 37°C with a heating pad (VI-20F, Fintronics Inc, Orange, CA). Concurrent recordings of striatal single-unit activity (B: −0.5 to 2.0 mm, L: 2.0 to 3.5 mm [44]) and cortical local field potentials (B: 3.0 to 4.0 mm, L: 1.5 to 2.2 mm lateral [44]) were obtained in all experiments. The signals were amplified (NeuroData, Delaware Water Gap, PA or Multiclamp 700B, Molecular Devices, Sunnyvale, CA), filtered (bandwidth 300–3000 Hz), digitized (Digidata 1322A, Molecular Devices) and acquired (Axoscope, Sunnyvale, CA) at a sampling rate of 20 kHz. The isolated single-unit was typically monitored for at least 5 min to ensure the stability of the firing rate, firing pattern, and spike waveform, followed by 10 min of baseline activity recording. All time series analyses were performed with Statistica 6 (Statsoft Inc., Tulsa, OK); the interspike interval was obtained by means of spike amplitude discrimination (Clampfit 10, Molecular Devices).

Striatal Microinjections

All surgical procedures performed before striatal microinjections were conducted as described above (see In vivo electrophysiology). A single stainless steel cannula (29-gauge, Plastics One Inc., ReanueX, VI) was first stereotaxically implanted in the dorsal striatum (B: 0.7 mm, L: 3.0 mm, V: −4.5 mm). At least 30 min after implantation, each rat received an intrastriatal infusion (0.1 μl/min for 10 min) of either vehicle (0.5% DMSO in aCSF) or ODQ (50 μM) and all cannula were left in place for 5 min after completion of the microinjection. The same cannula was then placed in a more caudal and lateral position (B: −0.3 mm), and an identical infusion of vehicle or ODQ was performed 20 minutes after completion of the first microinjection. The cannula was left in place for an additional 60 min after completion of the second microinjection, and brains were then extracted for cytochrome oxidase histochemistry.

Cytochrome oxidase histochemistry and densitometry measures

Histochemistry of CO-I was performed according to a modified protocol of Tseng et al (2006) [45]. Coronal sections (50 μm thick) containing the STN were mounted onto glass slides and incubated for 90 minutes at 37°C in 0.1 M PB (pH 7.4) containing 0.50 g/L of 3,3’-diaminobenzidine, 0.33 g/L of horse heart cytochrome c, 44 g/L of sucrose, and 0.2 g/L of catalase. After a 90- minute incubation, slides were rinsed, dehydrated and coverslipped. Stained sections were captured with a slide scanner (Coolscan IV; Nikon, Japan), and the mean relative optical density (ROD) per pixel was determined by subtracting the optical density of the background from that of the STN. Background OD was measured at the level of the internal capsule. For each animal, a single value per STN was obtained by averaging measurements from 4–6 sections.

MPTP model and stepping test

Ten-month old C57BL/6 male mice weighing 25–35 g were single housed with food pellets and water available ad libitum. The animal room was maintained at a constant temperature and humidity on a 12 hour light-dark cycle. All animals were acclimated to the animal facility for at least 3 weeks before their use. A baseline stepping performance was monitored in all animals for 2 to 3 days before the first injection, and at least 3 trials of the stepping test were obtained before they were randomly assigned to receive weekly injections of MPTP or vehicle. Each mouse received 2 injections per week (3.5 day intervals) of MPTP (20 mg/kg, s.c.) or vehicle for 6 weeks (12 injections), and subsequent changes in the number of adjusting steps were recorded. The stepping test was performed as previously described [25].

Tyrosine hydroxylase immunohistochemistry and dopamine cell counting

The extent of the DA lesion was estimated by means of TH immunohistochemistry performed on free-floating sections, as previously reported [25,27]. Briefly, serial coronal sections of 50 μm thick were obtained from the mesencephalon using a freezing microtome (SM-2000R; Leica Microsystems, Germany) and exposed to rabbit anti-TH for 72 hours. Sections were then incubated for 2 hours in biotinylated goat anti-rabbit IgG (1:500; Vector Laboratories, CA), and the bound antigen-antibody complexes were visualized with 3,3’-diaminobenzidine and H2O2 tablets dissolved in 0.05 M Tris, pH 7. The degree of the DA lesion was estimated by counting the number of TH positive neurons in the SN at four serial coronal sections (200 μm apart) using ImageJ (NIH, USA, http://rsb.info.nih.gov/ij/). The 4 stereotactic planes lie between −5.2 and −5.8 mm from bregma in rats and −3.1 to −3.7 mm from bregma in mice.

Statistical analyses

All measurements are expressed as mean ± SEM and the differences between experimental conditions were considered statistically significant when P<0.05. In cases where data were not normally distributed or had unequal variances, Kruskal-Wallis ANOVA by ranks was used for multiple comparisons involving interrelated proportions.

Acknowledgments

We thank Drs. Gloria Meredith and Amiel Rosenkranz for helpful comments, and Dan Thomas, Peter Campbell and Pascal Acocli for excellent technical assistance.

Author Contributions

Conceived and designed the experiments: KYT ARW. Performed the experiments: KYT AC NS AD DKG ES MJP SRB SS DJP ARW. Analyzed the data: KYT AC NS AD DKG DJP ARW. Wrote the paper: KYT ARW.

References

1. Stocchi F, Tagliati M, Olanow CW (2008) Treatment of levodopa-induced motor complications. Mov Disord 23 Suppl 3: S599–612.
2. West AR (2010) Chapter 10: Nitric Oxide Signaling in the Striatum; Steiner HaT K.Y., ed. USA: Handbook of Basal Ganglia Structure and Function; Academic Press/Elsevier. pp 187–200.
3. Garthwaite J (2008) Concepts of neural nitric oxide-mediated transmission. Eur J Neurosci 27: 2783–2802.
4. Hofmann M, Spano PF, Trabucchi M, Kumakura K (1977) Guanylate cyclase activity in various rat brain areas. J Neurochem 29: 395–396.
5. Matusoka I, Giulii G, Poyard M, Stengel D, Parma J, et al. (1992) Localization of adenyllyl and guanylyl cyclase in rat brain by in situ hybridization: comparison with cDNAs mRNA distribution. J Neurosci 12: 3350–3360.
6. Ariano MA (1983) Distribution of components of the guanosine 3’5’-phosphate system in rat caudate-putamen. Neuroscience 10: 707–723.
7. Ding JD, Burette A, Nedevtsiyi PI, Schmidt HH, Weinberg RJ (2004) Distribution of soluble guanylyl cyclase in the rat brain. J Comp Neurol 472: 437–448.

8. Galati S, D’Angelo V, Scarmati E, Stazione P, Martorana A, et al. (2008) In vivo electrophysiology of dopamine-denervated striatum: focus on the nitric oxide/cGMP signaling pathway. J Neurochem 62: 409–420.

9. Giorgi M, D’Angelo V, Esposito Z, Iaccarini V, Sorge R, et al. (2008) Lowered cAMP and cGMP signaling in the brain during levodopa-induced dyskinesias in hemiparkinsonian rats: new aspects in the pathogenetic mechanisms. Eur J Neurosci 28: 941–950.

10. Nishi A, Watanabe Y, Higashi H, Tanaka M, Nairn AC, et al. (2005) Glutamate regulation of DARPP-32 phosphorylation in neostriatal neurons involves activation of multiple signaling cascades. Proc Natl Acad Sci U S A 102: 1190–1204.

11. Ondracek JM, Dec A, Hoque KE, Lim SA, Rasoudi G, et al. (2008) Feed-forward excitation of striatal neuron activity by frontal cortical activation of nitric oxide signaling in vivo. Eur J Neurosci 27: 1739–1754.

12. Sammut S, Thrëllë S, West AR (2010) Nitric oxide-sensitive guanylyl cyclase signaling regulates corticostriatal transmission and short-term synaptic plasticity of striatal projection neurons recorded in vivo. Neuropharmacology 58: 624–631.

13. Tsou K, Snyder GL, Greenberg P (1993) Nitric oxide/cGMP pathway stimulates phosphorylation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, in the substantia nigra. Proc Natl Acad Sci U S A 90: 3462–3465.

14. Chalimoniuk M, Langford J (2007) The effect of subchronic, intermittent L-DOPA treatment on neuronal nitric oxide synthase and soluble guanylyl cyclase expression and activity in the striatum and midbrain of normal and MPTP-treated mice. Neurochem Int 50: 821–833.

15. Chalimoniuk M, Langford J, Lukacova N, Marsala J (2004) Upregulation of guanylyl cyclase expression and activity in striatum of MPTP-induced parkinsonism in mice. Biochem Biophys Res Commun 324: 118–126.

16. Thrëllë S, Sammut S, Menotti FS, Schmidt CJ, West AR (2009) Inhibition of Phosphodiesterase 10A Increases the Responsiveness of Striatal Projection Neurons to Cortical Stimulation. J Pharmacol Exp Ther 328: 785–795.

17. West AR, Grace AA (2004) The nitric oxide–guanylyl cyclase signaling pathway modulates membrane activity States and electrophysiological properties of striatal medium spiny neurons recorded in vivo. J Neurosci: 24: 1924–1935.

18. Tseng KY, Kasanetz F, Kangjianman R, Riquelme LA, Murer MG (2001) Cortical slow oscillatory activity is reflected in the membrane potential and spike trains of striatal neurons in rats with chronic nigrostriatal lesions. J Neurosci: 21: 6430–6439.

19. Garthwaite J, Southam E, Boulton CL, Nielsen EB, Schmidt K, et al. (1995) Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by HH-1,2,4oxadiazolo[4,3-a]quinolinol-1-one. Mol Pharmacol 48: 184–191.

20. Zhao Y, Brandish PE, DeValentini M, Schelvis JP, Babcock GT, et al. (2000) Inhibition of soluble guanylate cyclase by ODQ. Biochemistry 39: 10848–10854.

21. Mallet N, Ballion B, Le Moine C, Gonon F (2006) Cortical inputs and GABA neurons to Cortical Stimulation. J Pharmacol Exp Ther 328: 785–795.

22. Hirsch EC, Perier C, Orieux G, Francois C, Feger J, et al. (2008) Metabolic effects of nigrostriatal denervation in basal ganglia. Trends Neurosci: 23: 878–85.

23. Blandini F, Nappi G, Tassorelli C, Martignoni F (2000) Functional changes of the basal ganglia circuitry in Parkinson’s disease. Prog Neurobiol 62: 63–88.

24. Renabid AL, Chabardes S, Mitrofanis J, Pollak P (2009) Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson’s disease. Lancet Neurol 8: 67–81.

25. Blume SR, Cass DK, Tseng KY (2009) Stepping test in mice: a reliable approach in determining forelimb akinesia in MPTP-induced Parkinsonism. Exp Neurol 219: 208–211.

26. Olsson M, Niskiah G, Bentslage C, Bjorklund A (1995) Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J Neurosci: 15: 3883–3871.

27. Tseng KY, Kargieman L, Garcia S, Riquelme LA, Murer MG (2005) Consequences of partial and severe dopaminergic lesion on basal ganglia oscillatory activity and akinesia. Eur J Neurosci: 22: 2579–2586.

28. Deumens R, Blokland A, Prickaerts J (2002) Modeling Parkinson’s disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. Exp Neurol: 175: 303–317.

29. Roedter A, Winkler C, Samii M, Walter GF, Brandis A, et al. (2001) Comparison of unilateral and bilateral intrastratal 6-hydroxydopamine-induced axon terminal lesions: evidence for interhemispheric functional coupling of the two nigrostriatal pathways. J Comp Neurol: 439: 217–229.

30. Calabresi P, Gubellini P, Marfia GA, Pisani A, et al. (2000) Synaptic transmission in the striatum: from plasticity to neurodegeneration. Prog Neurobiol: 61: 231–265.

31. Calabresi P, Gubellini P, Gontzea D, Sancesario G, Morello M, et al. (1999) A critical role of the nitric oxide/cGMP pathway in corticostriatal long-term depression. J Neurosci: 19: 2499–2509.

32. Dorevitch N, Sergeeva OA, Yanesovych V, Chepkova AN, Selbach O, et al. (2003) Corticostriatal synaptic plasticity in endothelial nitric oxide synthase deficient mice. Brain Res: 964: 159–163.

33. Chepkova AN, Fliescher W, Kuzminetzek T, Dorevitch N, Haas HL, et al. (2009) Developmental alterations of DFFPQ-induced long-term depression of corticostriatal synaptic transmission: switch from NMDA receptor-dependent towards CB1 receptor-dependent plasticity. Pflugers Arch 459: 131–141.

34. O’Donnell P, Grace AA (1997) Cortical afferents modulate striatal gap junction permeability via nitric oxide. Neuroscience: 76: 1–5.

35. Sammut S, Park DJ, West AR (2007) Frontal cortical afferents facilitate striatal nitric oxide transmission in vivo via a NMDA receptor and neuronal NOS-dependent mechanism. J Neurochem: 103: 1145–1156.

36. Altar GA, Boyar WC, Kim HS (1990) Discriminatory roles for D1 and D2 dopamine receptor subtypes in the in vivo control of neostriatal cyclic GMP. Eur J Pharmacobiol: 181: 17–21.

37. Suriak JA, McCarthy SA, Chupin DS, Fujisawa RA, James LC, et al. (2006) Genetic deletion of the striatum-enriched phosphodiesterase PDE10A: evidence for altered striatal function. Neuropharmacology: 51: 374–383.

38. West AR, Grace AA (2002) Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. J Neurosci: 22: 294–304.

39. Colwell CS, Levine MS (1995) Excitatory synaptic transmission in neostriatal neuronal regulation by cyclic AMP-dependent mechanisms. J Neurosci: 15: 1704–1713.

40. Taga-Taga F, Merida E, Neitz A, Eyssel UT, Koessler D, et al. (2009) More than a retrograde messenger: nitric oxide needs two cGMP pathways to induce hippocampal long-term potentiation. J Neurosci: 29: 9344–9350.

41. Serulle Y, Zhang S, Ninan I, Puzzo D, McCarthy M, et al. (2007) A GluR1- cGKII interaction regulates AMPA receptor trafficking. Neuron: 56: 670–689.

42. Menotti FS, Faraci WS, Schmidt CJ (2006) Phosphodiesterases in the CNS: targets for drug development. Nat Rev Drug Discov: 5: 660–670.

43. Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev: 58: 480–520.

44. Paxinos G, Watson C (1998) The Rat Brain in Stereotaxic Coordinates. New York: Academic Press. 256 p.

45. Tseng KY, Aurin F, Lewis BL, O’Donnell P (2006) Altered prefrontal cortical metabolic response to mesocortical activation in adult animals with a neonatal ventral hippocampal lesion. Biol Psychiatry: 60: 585–590.

46. Jouvert P, Revel MO, Lazaris A, Aurin D, Langley K, Zsiller J (2004) Activation of the cGMP Pathway in Dopaminergic Structures Reduces Cocaine-Induced EGR-1 Expression and Locomotor Activity. J Neurosci: 24: 10716–10725.