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Nitric oxide-soluble guanylyl cyclase signaling regulates corticostriatal transmission and short-term synaptic plasticity of striatal projection neurons recorded *in vivo*

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Summary

Striatal medium-sized spiny neurons (MSNs) contain the highest levels of soluble guanylyl cyclase (sGC) in the brain. Striatal sGC signaling is activated by nitric oxide (NO) and other neuromodulators. MSNs also express cGMP-dependent protein kinase and other components of the cGMP signaling system which are critically involved in integrating corticostriatal transmission and regulating synaptic plasticity in striatal networks. However, the influence of tonic and phasic activation of this signaling pathway on striatal MSN activity is poorly understood. The present study examined the impact of systemic administration of the selective sGC inhibitor [1H-[1,2,4] oxadiazolo-[4,3-a] quinoxalin-1-one] (ODQ) on spike activity evoked using low and high frequency electrical stimulation of the frontal cortex. MSN activity was monitored using single-unit extracellular recordings in urethane-anesthetized rats. ODQ administration significantly decreased spike activity evoked by low frequency cortical stimulation in a stimulus intensity- and time-dependent manner. Additionally, ODQ administered along with the neuronal NO synthase inhibitor 7-nitroindazole (7-NI) potentially decreased the incidence of excitatory responses observed during high frequency train stimulation of the contralateral frontal cortex. The short-term depression of cortically-evoked spike activity induced by train stimulation was enhanced following pretreatment with ODQ in MSNs exhibiting an excitatory response during cortical train stimulation. Unexpectedly, this effect of ODQ was reversed in animals receiving co-administration of ODQ and 7-NI. 7-NI/ODQ co-administration also reversed measures of short-term depression observed in MSNs exhibiting an inhibitory response during cortical train stimulation. These observations extend previous studies showing that tonic and phasic NO-sGC signaling modulates the responsiveness of MSNs to corticostriatal input. Moreover, phasic activation of NO signaling is likely to regulate short-term changes in corticostriatal synaptic plasticity via complex mechanisms involving both sGC-cGMP-dependent and independent pathways.

Keywords

striatum; nitric oxide synthase; cyclic nucleotides; short-term depression; electrophysiology

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Introduction

Dysfunctional neurotransmission in frontostriatal pathways has been shown to be critically involved in the pathophysiology of many brain disorders. Information transmitted via these glutamatergic afferents is integrated in functionally coupled networks of striatal principal neurons and interneurons (Wilson, 2004). The vast majority (~95%) of striatal neurons are medium-sized spiny neurons (MSNs) which can be distinguished from striatal interneurons based on their unique morphological and physiological properties (Kawaguchi, 1997; Bolam *et al.*, 2000; Tepper *et al.*, 2004). Striatal MSNs have been shown to receive synaptic inputs from nitric oxide (NO) producing interneurons (Sancesario *et al.*, 2000; Hidaka & Totterdell, 2001; French *et al.*, 2005). These synaptic inputs contain the neuronal isoform of NO synthase (nNOS) and terminate on the shafts of dendritic spines known to express high levels of soluble guanylyl cyclase (sGC), cGMP, cGMP-dependent protein kinase (PKG), and cyclic nucleotide phosphodiesterases (PDEs) (Ariano *et al.*, 1982; Ariano, 1983; Matsuoka *et al.*, 1992; Furuyama *et al.*, 1993; Fujishige *et al.*, 1999; Ding *et al.*, 2004; Coskran *et al.*, 2006). Numerous recent reports have demonstrated an important role for striatal NO-sGC signaling in the generation of spontaneous and psychostimulant-driven motor behavior (see Del Bel *et al.*, 2005 for review). Thus, further characterization of sGC-cGMP signaling is critical for understanding normal striatal function and pathophysiological conditions such as Parkinson's disease (PD) and addiction.

NO-mediated activation of striatal MSNs is at least partially dependent upon sGC-cGMP signaling mechanisms (Greengard, 2001). Intrastriatal infusion of membrane permeable cGMP analogues and intracellular injection of cGMP into MSNs has been shown to depolarize the cell membrane and facilitate cortically-driven activity (West & Grace, 2004). Similar effects are observed following local or systemic administration of inhibitors targeting PDEs which act to metabolize cGMP and cAMP (West & Grace, 2004; Threlfell *et al.*, 2008). Numerous findings also indicate that striatal sGC-cGMP signaling cascades play a key role in the regulation of synaptic plasticity (Calabresi *et al.*, 1999a; Calabresi *et al.*, 1999b; Calabresi *et al.*, 2000), protein kinase and protein phosphatase activities (Walaas & Greengard, 1984; Nishi *et al.*, 2005), and gene expression (Konradi *et al.*, 1994; Berke *et al.*, 1998). Thus, disruption of striatal NO-cGMP signaling cascades results in profound changes in behavioral, electrophysiological, and molecular responses to pharmacological manipulations of dopamine and glutamate transmission (Greengard, 2001; West *et al.*, 2002; Del Bel *et al.*, 2005; Calabresi *et al.*, 2007).

Given the above, the sGC-cGMP signaling pathway may contain viable targets for pharmacotherapies aimed at treating brain disorders characterized by dysfunctional excitatory transmission and/or dysregulation of dopaminergic modulation. In support of this, agents that enhance striatal levels of cGMP or PKG activity have been shown to inhibit exploratory locomotor behaviors, conditioned avoidance responding, and locomotor activity in rodents induced by cocaine, amphetamine and other psychostimulants (Jouvert *et al.*, 2004; Siuciak *et al.*, 2006a; Siuciak *et al.*, 2006b; Schmidt *et al.*, 2008). Inhibitors of cyclic nucleotide metabolism also potentiate haloperidol-induced catalepsy (Kostowski *et al.*, 1976; Siuciak *et al.*, 2006a). These biochemical and behavioral effects resulting from augmentation of sGC-cGMP signaling are consistent with enhanced striatal MSN activation and increased striatal output.

The goal of the current study was to characterize the effect of nNOS and sGC inhibition on corticostriatal signaling and short-term synaptic plasticity in MSNs recorded in the intact animal. Thus, we examined the impact of systemic administration of a selective sGC inhibitor, administered alone or in combination with a selective nNOS inhibitor, on cortically-evoked

spike activity. A portion of these results have been presented in preliminary form (Threlfell & West, 2007).

Methods

Drugs

Urethane, 7-nitroindazole (7-NI), and Cremophor EL were purchased from Sigma Chemical (St. Louis, MO, USA), 1H-[1,2,4] oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) was purchased from Tocris Bioscience (Ellisville, MO, USA). All other reagents were of the highest grade commercially available.

Subjects and surgery

Electrophysiological recordings were made from 51 male Sprague-Dawley (Harlan, Indianapolis, IN) rats weighing 241–387 grams. Prior to use, animals were housed two- or three-per cage 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 animal protocols were approved by the Rosalind Franklin University of Medicine and Science Institutional Animal Care and Use Committee and adhere to the *Guide for the Care and Use of Laboratory Animals* published by the USPHS. Prior to surgery, animals were deeply anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic apparatus (Narishige International USA Inc., East Meadow, NY or David Kopf Instruments, Tujunga, CA). The level of anesthesia was periodically verified via the hind limb compression reflex and maintained using supplemental administration of anesthesia as previously described (Sammut *et al.*, 2006; Sammut *et al.*, 2007a; Sammut *et al.*, 2007b; Ondracek *et al.*, 2008). Temperature was monitored using a rectal probe and maintained at 37°C using a heating pad (VI-20F, Fintronics Inc, Orange, CT). To minimize pain or discomfort, a solution of lidocaine HCl (2%) and epinephrine (1:100,000) (Henry Schein, Melville, NY) was injected into the scalp (s.c.) in a volume of ~0.3ml and allowed to diffuse for several minutes. An incision (~2–4 cm) was then made in the scalp and burr holes (~2–3 mm in diameter) were drilled in the skull overlying the right hemisphere of the frontal cortex (coordinates: 3.0–4.0 mm anterior from bregma, 1.5–2.2 mm lateral from the midline) and dorsal striatum (coordinates: –0.5–2.0 mm anterior from bregma, 2.0–3.5 mm lateral from the midline). In experiments involving train stimulation of the contralateral cortex, an additional burr hole was made overlying the left hemisphere of the frontal cortex (coordinates: 3.0–4.0 mm anterior from bregma, 1.5–2.2 mm lateral from the midline). In experiments involving antidromic stimulation of the substantia nigra pars reticulata (SNr), an additional burr hole was made overlying the right hemisphere of the SNr (coordinates: 5 mm posterior from bregma, 2.5 mm lateral from the midline). The dura mater was resected and the stimulating and recording electrodes were lowered into the brain using a Narishige or Kopf micromanipulator. All coordinates were determined using a rat brain stereotaxic atlas (Paxinos & Watson, 1986).

Electrical stimulation and antidromic activation

Concentric bipolar stimulating electrodes were implanted in the frontal cortex ipsilateral and/or contralateral (in the case of train stimulation) to the recording electrode as indicated below and previously shown (Ondracek *et al.*, 2008). In some of the experiments involving cortical train stimulation, neurons were also tested using antidromic stimulation (Table 1). In this subset of experiments an additional concentric bipolar stimulating electrode was implanted ipsilaterally in the SNr (coordinates were within the same range as indicated above). Electrical stimuli with durations of 500 µs and intensities between 0.25–1.5 mA were generated using a stimulator and photoelectric constant current/stimulus isolation unit (S88 stimulator with PSIU6F stimulus isolation unit, Grass Instruments, Quincy, MA) and delivered in single pulses (0.5 Hz) for duration of 200 seconds as previously described (Ondracek *et al.*, 2008). In studies

involving high frequency stimulation of the contralateral cortex, stimulus trains (500 μ A, 30 Hz, 1000 ms train duration, 2 sec inter-train interval (ITI)) were delivered for duration of 50 seconds. The ITI is defined as the time between the onset of the first stimulus pulse in the initial train and the first stimulus pulse in the subsequent train. The duration and pattern of the stimulation train were designed to approximate the natural burst firing activity (spikes per burst, intraburst frequency, and bursts per second) of corticostriatal pyramidal neurons (Cowan & Wilson, 1994) and cortical neurons recorded in behaving animals (Steriade, 2001). In a subset of studies, antidromic stimulation of the SNr was used to identify striatonigral projection neurons (SNr+). The following criteria were used to determine if spikes evoked by SNr stimulation were antidromic in nature: (1) constant latency of observed spike response, (2) all-or-none property of antidromic spikes as determined using sub- and supra-threshold stimulus intensities, and (3) collision of the antidromic spikes with cortically-evoked orthodromic spikes (see Figure 1C and Ondracek et al., 2008). Collisions which could be observed consistently over 10 consecutive trials were considered positive. Striatal neurons were considered unresponsive to antidromic stimulation of the SNr if their spike responses evoked by SNr stimulation did not meet the above criteria or if they exhibited no spike response to SNr stimulation when tested utilizing a maximal current intensity (1.5 mA).

Extracellular recordings

Extracellular recording microelectrodes were manufactured from 2.0 mm o.d. borosilicate glass capillary tubing (WPI, New York, NY) using a vertical micropipette puller (model PE-21, Narishige) and recordings of cortically-evoked activity were performed as previously described (Ondracek et al., 2008; Threlfell et al., 2009). In within-subjects studies, the impact of varying the intensity of electrical stimuli within a given range of current (0.8–1.2 mA) on evoked spike activity was assessed over 50 stimuli per trial (Figure 1A). In between-subjects studies, stimulation currents of individual test pulses were titrated to an intensity (250–1500 μ A) which reliably evoked spike activity approximately 50% of the time (Ondracek et al., 2008). A pre-train stimulation baseline trial consisting of 100 individual single-pulse stimulations delivered over 200 sec was then recorded. Once stable levels of single-pulse evoked spiking were obtained, a series of train stimulations (25) were delivered to the contralateral cortex (500 μ A, 30 Hz, 1000 ms train duration, 2 sec ITI for duration of 50 seconds; Figure 1B). Immediately following the train stimulation trial, three additional post-train stimulation trials (200 sec each, post-1, post-2 and post-3) were recorded as previously described (Ondracek et al., 2008). Lastly, in a subset of the above studies involving cortical train stimulation, activation of the SNr was performed to determine whether the recorded cell exhibited antidromic responses as described above. In studies involving systemic administration of drugs, vehicle (10% Cremophor EL in 0.9% saline), ODQ (10 mg/kg), or ODQ and 7-NI (50 mg/kg) was injected at least 10 minutes prior to the recording session. The effective doses of ODQ and 7-NI were derived from previous studies (Chan et al., 2004; Ergun & Ergun, 2007; Ondracek et al., 2008).

Data Analysis and Statistics

Firing rate histograms were constructed (1.0 ms bins of 2 min epochs) from cells recorded following administration of either vehicle, ODQ, or ODQ+7-NI. Peri-stimulus time histograms were constructed (1.0 ms bins) for each cortical stimulation trial and spike probabilities were calculated by dividing the number of evoked action potentials (either 0 or 1 per pulse) by the number of stimuli delivered (West & Grace, 2000; Ondracek et al., 2008). Single unit and group data were summarized using population PSTHs, spike probability, spike latency and S.D. latency plots as indicated. Excitatory (E) responses observed during train stimulation were operationally defined as an increase in spike probability of > 2 S.D. above the pre-train stimulation mean. Inhibitory (I) responses observed during train stimulation were operationally defined as a decrease in spike probability of > 2 S.D. below the pre-train stimulation mean

(Ondracek *et al.*, 2008). The statistical significance of drug-induced changes in spike activity was determined using either a paired t-test, Fisher exact test or one/two-way analysis of variance (ANOVA) with one factor repetition as indicated (Sigma Stat, Jandel). Also, Bonferroni or Tukey post-hoc tests were used as indicated to determine which group(s) contributed to overall differences seen with ANOVA.

Histology

After completion of each experiment, rats were deeply anesthetized and perfused transcardially with ice-cold saline followed by 10% formalin in buffered phosphate (PB) (EMS, Hatfield, PA). Brains were removed and postfixed in formalin/sucrose solution (30%) and stored at 4° C until saturated. Brains were then sectioned into 50 µm coronal slices, mounted, and stained with Neutral red/Cresyl Violet (10:1) solution to enable histological assessment of stimulating and recording electrodes (Sammur *et al.*, 2007b).

Results

Stimulating and recording electrode placements

All identified stimulating electrode tips were confirmed to lie in the frontal cortex between 3.2 and 4.7 mm anterior to bregma, 1.2 and 3.0 mm lateral to the midline, and 2.0 and 4.4 mm ventral to the skull (Paxinos & Watson, 1986). In studies involving antidromic stimulation of the SNr, stimulating electrode tips were confirmed to lie between 4.5 and 6.0 mm posterior to bregma, 1.2 and 3.0 mm lateral to the midline, and 7.4 and 8.5 mm ventral to the dural surface (Paxinos & Watson, 1986). Identified placements for recording electrodes implanted into the striatum were verified to lie between 0.3 posterior and 1.7 mm anterior to bregma, 2.0 and 3.6 mm lateral to the midline, and 3.4 and 7.5 mm ventral to the dural surface (Paxinos & Watson, 1986).

Impact of sGC inhibition on responsiveness of striatal neurons to low frequency stimulation of the frontal cortex

To assess the impact of sGC activity on cortically-evoked spike activity, striatal neurons were recorded before and after (every 10 mins up to 50 mins) systemic administration of vehicle (10% Cremophor EL, in 0.9% saline, i.p.) or the selective sGC inhibitor ODQ (10 mg/kg, i.p.). Single-units were isolated using single-pulse stimulation of the ipsilateral cortex (Figure 1A). Striatal neurons exhibiting spike characteristics resembling cholinergic (tonic or regular firing at a rate of ~1–4 Hz) or fast-spiking interneurons (respond to low intensity cortical stimulation with a high-frequency train of short duration (<0.9 ms) action potentials) (Kawaguchi, 1997; Mallet *et al.*, 2005) were not included in the current data set. Additionally, a subset of neurons in vehicle- and drug-treated groups was tested for antidromic responses following administration of the train stimulation protocol (see Methods, Figure 1C). No difference in the proportion of SNr+ cells was observed between vehicle-, ODQ-, or ODQ+7-NI-treated groups (Table 1). All neurons recorded in this study exhibited spike characteristics which were consistent with the SNr+ cells identified in this and other studies (Mallet *et al.*, 2005; Mallet *et al.*, 2006; Threlfell *et al.*, 2008). Thus, it is highly likely that the data set reported herein is derived exclusively from recordings of MSNs.

In within-subjects studies, successful recordings of evoked activity before and after vehicle or ODQ administration were obtained for n = 5 and n = 13 cells/rats, respectively. Individual stimulation trials consisting of 50 single pulses each (0.5 Hz, 500 µs) were delivered to the frontal cortex using multiple current amplitudes (0.8, 1.0, 1.2 mA). Short latency spike activity (<20 ms) evoked during repeated single-pulse stimulation of the ipsilateral frontal cortex was dependent on the level of current intensity following systemic administration of vehicle and ODQ (Figures 1,2; ($F_{(2,22)} = 31.014$, $p < 0.001$; two-way ANOVA). Interestingly, systemic

administration of ODQ (10 mg/kg) induced a decrease in cortically-evoked spike activity at higher stimulus intensities (1.2 mA) in a time-dependent manner (Figure 2A,B; $F_{(5, 45)} = 2.812$, $p < 0.05$; two-way ANOVA, 20 minutes post ODQ: -24%; 30 minutes post ODQ: -18%; 50 minutes post ODQ: -16%) compared to pre-drug controls. In addition, within-group comparisons of the onset latency of cortically-evoked spikes also revealed a strong trend towards an increase in this measure at higher stimulus intensities (1.2 mA) following systemic administration of ODQ (Figure 2C; $F_{(5, 45)} = 2.262$, $p = 0.064$; two-way ANOVA). No significant difference in the S.D. of spike latency of cells was observed following systemic administration of ODQ as compared to pre-drug controls (data not shown).

Impact of sGC inhibition on responsiveness of striatal neurons to high frequency stimulation of the frontal cortex

Afferents from the contralateral frontal cortex were activated using electrical stimuli delivered for 50 s as trains (25 individual trains) of high-frequency stimulation (500 μ A, 30 Hz, 1000 ms train duration, 2 s ITI, see Methods and (Ondracek et al., 2008)). This stimulation paradigm has also previously been shown by our lab to elicit robust phasic NO signals in the striatum which are attenuated by systemic and intrastriatal administration of nNOS inhibitors and NMDA receptor antagonist (Sammut et al., 2007b; Ondracek et al., 2008; Park et al., 2009).

All recordings were performed ≥ 10 min after administration (i.p.) of vehicle, ODQ (10 mg/kg, i.p.), or ODQ and the selective nNOS inhibitor 7-NI (ODQ+7-NI, 50 mg/kg, i.p.). The mean $\pm$ S.E.M. time of recording post-injection was similar across groups ($p > 0.05$, vehicle: 67 ± 10 min; ODQ: 54 ± 6 min, ODQ+7NI: 64 ± 6 min). In a subgroup of rats, the effects of the above train stimulation protocol on 11 cells were recorded prior to i.p. vehicle or drug administration (control cells). Data recorded from these cells was compared to similar data from recordings performed in vehicle treated animals and no significant differences in current required to evoke spike activity approximately 50% of the time were observed between groups ($p > 0.05$, data not shown). Therefore the data were pooled and are represented in all results/figures as a single vehicle treatment group.

As previously reported (Ondracek et al., 2008), in the majority of striatal neurons recorded in vehicle treated rats, train stimulation of the contralateral frontal cortex induced either an excitation (E response) or an inhibition (I response) of cortically-evoked spike activity (Table 2, Figure 3A). The spike activity of a minority of cells recorded during the train stimulation trial in vehicle treated rats (~25%) did not change (NR response) (Table 2). Between group comparisons showed that ODQ administration did not affect the frequency of E, I and NR responses (Table 2, $p > 0.05$, Fisher exact test). Interestingly, a significant decrease in the E response type was observed in animals treated with ODQ+7-NI (Table 2, $p < 0.05$, Fisher exact test), an effect which was associated with a significant increase in the incidence of the NR response type (Table 2, $p < 0.05$, Fisher exact test).

To determine the effects of ODQ, administered alone or in combination with 7-NI, on train-induced changes in cortically-evoked activity, between group comparisons of spike activity were performed on neurons exhibiting E and I responses. Measures were assessed prior to train stimulation (pre-train), during train stimulation (train), and following (post 1–3) train stimulation (Figures 3–5). Analysis of cortically-evoked activity observed in vehicle, ODQ, and ODQ+7-NI treated rats revealed a significant main effect of train stimulation of the contralateral cortex across time ($F_{(4, 328)} = 4.904$, $p < 0.001$; two-way ANOVA). Consistent with our previous studies (Ondracek et al., 2008), following vehicle administration, train stimulation of the contralateral cortex was observed to induce a short-term depression (STD) of cortically-evoked activity (Figure 3B; $p < 0.005$). As shown in Figure 3C, between group comparisons of the effects of train stimulation across time on spike activity observed in neurons exhibiting E and I responses revealed significant main effects associated with response type

($F_{(1, 84)} = 40.105$, $p < 0.001$; two-way ANOVA) and train stimulation ($F_{(4, 84)} = 10.854$, $p < 0.001$; two-way ANOVA). Furthermore, significant interactions were observed between response type and train stimulation ($F_{(4, 84)} = 43.024$, $p < 0.001$), indicating that the magnitude of the observed train-induced effects was different in neurons exhibiting E and I responses. In support of this, pairwise comparisons of E and I responses revealed that recovery from train stimulation-induced STD of cortically-evoked spike activity varied according to the response type and was more rapid and complete in neurons responding to train stimulation with an E response (Figure 3C; $p < 0.05$ for all post-train trials). Interestingly, ODQ administration decreased cortically-evoked activity in E cells only in post-train stimulation trial 2 (Figure 4a; $p < 0.05$) without affecting cortically-evoked activity during train or post-train stimulation trials 1 and 3 (Figure 4a; $p > 0.05$). Co-administration of ODQ with 7-NI did not affect cortically-evoked activity during train stimulation of the contralateral cortex or subsequent post-stimulation responses in neurons responding to train stimulation with an E response (Figure 4a; $p > 0.05$). However, significant differences in spike probability were observed between ODQ and ODQ+7-NI groups for post-train trial 1 and 2 (Figure 4a; $p < 0.05$). ODQ administration did not affect cortically-evoked activity observed during train stimulation of the contralateral cortex or subsequent post-stimulation responses in neurons responding to train stimulation with an I response (Figure 4b; $p > 0.05$). However, significant differences in spike probability were observed between vehicle and ODQ+7-NI groups in post-train stimulation trial 1 (Figure 4b; $p < 0.05$). No significant differences in average onset latency of evoked spikes or standard deviation of spike latency were observed between vehicle, ODQ, and ODQ+7-NI groups (data not shown).

Discussion

The current studies show that tonic and phasic NO-sGC signaling activity acts to modulate cortically-driven spike activity and short-term synaptic plasticity of striatal MSNs. Thus, systemic administration of the sGC inhibitor ODQ significantly attenuated spike activity evoked by low frequency cortical stimulation and increased the STD of cortically-evoked activity observed following excitatory responses to train stimulation of the cortex. Consistent with our previous work using the nNOS inhibitor 7-NI (Ondracek *et al.*, 2008), co-administration of ODQ and 7-NI was observed to decrease the frequency of excitatory responses evoked during cortical train stimulation. However, the current study also revealed that some effects of ODQ were reversed in animals receiving co-administration of ODQ and 7-NI. These unexpected findings suggest that NO modulates synaptic plasticity across corticostriatal synapses via sGC-dependent and -independent processes which can function in opposition to each other. The implications of these findings are discussed below in more detail.

Regulation of striatal neuron activity by tonic NO- sGC-cGMP signaling

Steady-state levels of “tonic” cGMP are maintained in many brain regions including the hippocampus (Hopper & Garthwaite, 2006), cerebellum (Hopper *et al.*, 2004), vestibular nucleus (Podda *et al.*, 2004) and striatum (Globus *et al.*, 1995). In the striatum, genetic disruption (Siuciak *et al.*, 2006b) or pharmacological inhibition (Globus *et al.*, 1995) of nNOS activity decreases tonic cGMP levels by approximately 50%, indicating that tonic NO signaling is the major activator of sGC under non-stimulated conditions. Most studies indicate that tonic NO-sGC signaling facilitates neuronal activity and may couple this activity to increased blood flow and metabolic support (Estrada & DeFelipe, 1998). In support of this, the current study revealed that systemic application of the potent and selective sGC inhibitor ODQ decreased the responsiveness of striatal neurons to low frequency cortical stimulation. We have also recently shown that systemic administration of the nNOS inhibitor 7-NI strongly inhibited the spontaneous firing activity of striatal neurons isolated during low frequency cortical stimulation (Ondracek *et al.*, 2008). Moreover, disruption of endogenous NO-sGC signaling

via intrastriatal or intracellular application of antagonists/inhibitors was found to decrease the amplitude of depolarized up states and the responsiveness of MSNs to depolarizing and hyperpolarizing current steps (West & Grace, 2004). Importantly, the effects of NO-sGC inhibitors on membrane activity were, in many cases reversed by co-application of cGMP analogues, indicating that tonic cGMP signaling regulates the function of ion channels involved in controlling the membrane excitability of MSNs and their responsiveness to depolarizing stimuli (West & Grace, 2004).

Regulation of cortically-evoked activity by phasic NO-sGC-cGMP signaling

We have recently examined the impact of phasic NO signaling, evoked using the 30 Hz train stimulation paradigm described herein, on striatal oscillations using dual NO microsensor and local field potential recordings carried out concurrently in the contralateral and ipsilateral striatum, respectively (Sammut *et al.*, 2007b). These studies revealed that systemic administration of the non-specific NOS and sGC inhibitor methylene blue simultaneously attenuated evoked NO efflux and the peak frequency of local striatal field potential oscillations (Sammut *et al.*, 2007b). These findings indicate that nitrenergic transmission is strongly activated by convergent and synchronous burst firing of corticostriatal neurons. Phasic activation of NO-cGMP signaling may act to amplify synchronized corticostriatal transmission and entrain striatal output to cortical activity (Sammut *et al.*, 2007b). Moreover, disruption of nitrenergic transmission via sGC inhibition is likely to decrease the synchrony of local field potentials and depress the responsiveness of MSN networks to corticostriatal-dependent changes in synaptic efficacy.

As observed in our previous studies (Ondracek *et al.*, 2008), the 30 Hz train stimulation protocol had both excitatory and inhibitory effects on spike activity of MSNs assessed during the stimulation trial, but consistently induced a STD of cortically-evoked activity which was most prominent in the first post-train stimulation trial. Inhibition of nNOS activity decreased excitatory responses and cortically-evoked activity recorded during the train stimulation trial and enhanced the STD of spike activity induced by train stimulation (Ondracek *et al.*, 2008). Additional studies using the dopamine D2 receptor antagonist eticlopride indicated that the STD of cortically-evoked spike activity observed during post-train stimulation trials was mediated via D2 receptor activation (Ondracek *et al.*, 2008).

In the current study, inhibition of sGC activity using ODQ alone did not alter the incidence of excitatory or inhibitory responses observed during the train stimulation trial (Table 2), but did affect the magnitude of the STD observed following excitatory responses (Figure 4a). The lack of effect of ODQ on the incidence of excitatory or inhibitory responses was unexpected given the above mentioned outcomes from our previous studies using 7-NI (Ondracek *et al.*, 2008) and the current findings in animals administered ODQ+7-NI (Table 2). These findings suggest that in addition to sGC, NO signaling may act on other (ODQ insensitive) effector targets to control the responsiveness of MSNs to cortical input during ongoing excitatory transmission and following activity-dependent changes in synaptic efficacy. Based on previous studies (West & Galloway, 1997; West & Grace, 2004), we speculate that these ODQ-insensitive effects of NO may be occurring via presynaptic facilitation of glutamate release. Thus, NO signaling, stimulated via exogenous or endogenous sources of NO, has been shown to increase striatal extracellular glutamate levels and increase the amplitude and duration of cortically-evoked EPSPs and paired-pulse facilitation ratios (West *et al.*, 2002; West & Grace, 2004). These observations are consistent with studies using hippocampal synaptosomes which have shown that NO facilitates the release of synaptic vesicles directly in a calcium-independent manner via a s-nitrosylation mechanism (Meffert *et al.*, 1994; Meffert *et al.*, 1996). This postsynaptic modification by NO presumably promotes the docking and fusion of synaptic

vesicles and exocytosis of glutamate and may have contributed to the E responses observed during the train stimulation trial in our studies.

Unexpectedly, the combined effects of nNOS and sGC inhibition on STD of cortically-evoked spike activity observed in the current study were quite different than those observed in our previous study following inhibition of nNOS activity alone (Ondracek *et al.*, 2008). A significant difference between studies is that the inhibitory effects of ODQ were only observed in the subpopulation of cells which exhibited an excitatory response during the train stimulation trial. In contrast to sGC inhibition, nNOS inhibition abolished the excitatory response (Ondracek *et al.*, 2008), thus we cannot compare outcomes across excitatory groups in these studies. In our previous studies, nNOS inhibition facilitated the train-induced STD in the subpopulation of cells which exhibited an inhibitory response during the train stimulation trial (Ondracek *et al.*, 2008). The current observations that co-administration of ODQ and 7-NI reversed the train-induced STD in this same subpopulation of cells suggests that sGC-cGMP signaling facilitates STD in these cells, whereas an alternative NO signaling mechanism such as that discussed above, opposes this sGC-dependent effect. Indeed, the impact of NO-sGC modulation on MSN activity is likely to be complex and may depend on multiple factors including the MSN subtype (striatonigral versus striatopallidal), steady-state membrane potential of the neuron, ongoing synaptic activity across excitatory and inhibitory synapses, mode of NO transmission (i.e., tonic versus phasic), and the relative contribution of NO effector pathways involved in mediating the nitrgic modulation.

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Figure 1. Extracellular recording protocols and representative traces of single-unit activity

A) Representative traces of cortically-evoked spike activity of a single striatal unit recorded following systemic administration of vehicle. In each case, ten superimposed traces of cortically evoked spike responses from a single striatal neuron recorded under control conditions are shown for each stimulus intensity (0.8, 1.0, and 1.2 mA). Arrow indicates the location of the stimulus artifact. **B)** Left: six consecutive overlaid traces of cortically evoked responses elicited by single-pulse stimulation (500 μ A, 30 Hz, 1000 ms train duration, 2 s ITI, 0.5 ms) of the contralateral frontal cortex. Right: magnification of cortically-evoked spike responses shown on the left. Note that this unit responded to all ipsilateral cortical stimulations over 25 train stimulation trials. **C)** Representative recordings (10 overlaid traces) of orthodromic responses evoked via cortical stimulation (1), followed by constant latency responses to SNr stimulation (2). The collision test (3) is used to identify a striatonigral (SNr +) neuron by antidromic activation. The SNr stimulation was delivered either 40 ms (no collision) or 15–20 ms (collision) after the delivery of the stimulus pulse to the frontal cortex (Mallet *et al.*, 2005; Threlfell *et al.*, 2009). Notice the antidromic spikes evoked by SNr stimulation exhibited fixed onset latencies, whereas the onset latency of cortically-evoked orthodromic spikes was more variable.

Figure 2. Effects of systemic ODQ administration on the responsiveness of striatal neurons to single pulse electrical stimulation of the frontal cortex

A) A significant increase in spike probability was observed with increasing cortical stimulation intensity both prior to and following systemic administration of ODQ ($p < 0.001$; two-way ANOVA). Cortical stimulation produced a significant decrease in spike probability following ODQ administration at the 1.2 mA stimulation intensity ($**p < 0.01$; two-way ANOVA with Bonferroni t-test; $n = 8-13$ cells). **B)** A decrease in cortically-evoked spike activity, elicited using the 1.2 mA current intensity, was observed following ODQ administration at 20, 30, and 50 mins post-drug ($^{\#}p = 0.07$; $*p < 0.05$; one-way RM-ANOVA with Bonferroni t-test; $n = 8-13$ cells). **C)** ODQ administration increased the mean onset latency of cortically-evoked spikes (1.2 mA) ($^{\#}p = 0.064$; one-way RM-ANOVA; $n = 8-13$ cells, bars indicate S.E.M.).

Figure 3. Inhibitory and facilitatory effects of cortical train stimulation on cortically-evoked spike activity

A) Left: representative PSTHs showing the excitatory (E) response of a single striatal neuron to ipsilateral cortical stimulation recorded before (pre-train), during (contra-lateral train) and after (post 1–3) train stimulation of the contralateral cortex. **Right:** representative PSTHs showing the inhibitory (I) response of a single striatal neuron to ipsilateral cortical stimulation recorded before (pre-train), during (contra-lateral train) and after (post 1–3) train stimulation of the contralateral cortex. Each histogram was generated from 25–100 stimulation trials (50–200 s each) as indicated. Dashed vertical lines indicate the onset of single-pulse electrical stimulation. **B)** When data from all cells recorded in vehicle-treated animals were pooled (E, I, and NR responses), train stimulation of the contralateral cortex was found to produce variable effects across time, with the exception of the post-1 trial. Thus regardless of the response type exhibited during the train stimulation trial, a consistent depression of cortically-evoked activity was observed in the post-1 trial (* $p < 0.05$ as compared to pre-train spike probability; $n = 31$ cells/15 rats). **C)** Between-group comparisons of cortically-evoked spike activity measured prior to train stimulation (pre-train), during train stimulation (train), and following train stimulation (post 1–3) revealed significant differences between E and I responses across stimulus trials ($p < 0.001$). The spike probability of E response cells significantly increased during train stimulation of contralateral cortex (### $p < 0.001$). Cells responding to train stimulation with an E response also exhibited a significant decrease in cortically-evoked spike activity recorded in the first post-train stimulation trial (pre-train vs. post-1 spike probability; ## $p < 0.01$). This was followed by a rapid recovery of spike activity in post-2 and post-3 trials to levels that were not significantly different from pre-train values ($p > 0.05$). All E response data were derived from $n = 12$ cells/8 rats. Relative to pre-train levels, the spike probability of I response cells

significantly decreased during train stimulation of the contralateral cortex and remained depressed during the post-1, post-2, and post-3 stimulation trials ($^+p=0.06$; $^{\#}p<0.05$; $^{###}p<0.001$). All I response data were derived from $n=11$ cells/6 rats. The spike probability of I response cells was significantly lower than that of E response cells during train and in all post-train trials ($^*p<0.05$; $^{***}p<0.001$). All recordings of I and E responses are derived from vehicle-treated/control animals. All data were analyzed using a two-way ANOVA and Tukey post-hoc test.

Figure 4. Differential effects of ODQ administration and co-administration of ODQ and 7-NI on the responsiveness of striatal neurons to high frequency electrical stimulation of the contralateral frontal cortex

A) Systemic administration of ODQ did not alter cortically-evoked activity (ipsilateral stimulation) recorded in neurons responding to contralateral train stimulation with an E response during the train or post-1 and post-3 stimulation trials ($p > 0.05$). ODQ treatment did produce a significant inhibition of cortically-evoked activity recorded during the post-2 stimulation trial ($^{\#}p < 0.05$). Co-administration of ODQ and 7-NI did not affect cortically-evoked activity recorded during train stimulation or post-1, post-2, and post-3 stimulation trials as compared to vehicle-treated controls ($p > 0.05$). However, a significant reversal of the ODQ-induced effect was observed when 7-NI was co-administered with ODQ ($*p < 0.05$). **B)** ODQ administration did not affect cortically-evoked activity (ipsilateral stimulation) recorded in

neurons responding to contralateral train stimulation with an I response during the train or post-1, post-2, and post-3 stimulation trials ($p>0.05$). Co-administration of ODQ and 7-NI did not affect cortically-evoked activity recorded during train stimulation or post-2, and post-3 stimulation trials as compared to vehicle-treated controls ($p>0.05$). However, a significant reversal of the train-induced STD of cortically-evoked activity was observed when 7-NI was co-administered with ODQ ($*p<0.05$). All data were derived from $n=12-23$ cells/6–14 rats. All data were analyzed using a two-way ANOVA and Tukey post-hoc test.

Table 1

Effect of ODQ administered alone and together with 7-NI on the relative proportion of striatal neurons exhibiting antidromic spikes in response to SNr stimulation

Response	Treatment		
	Control	ODQ	ODQ + 7NI
SNr+	40.0% (6/15 cells)	23.1% (3/13 cells)	19.0% (4/21 cells)
SNr-	60.0% (9/15 cells)	76.9% (10/13 cells)	81.0% (17/21 cells)

Ratios in parentheses indicate the number of cells exhibiting the indicated type of response per number of cells tested. There was no difference between the proportion of SNr+ cells recorded following systemic ODQ or vehicle administration ($p>0.05$; Fisher exact test).

Table 2

Effects of ODQ administered alone and together with 7-NI on the observed frequency of excitatory (E), inhibitory (I), and no response (NR) responses of striatal cells during cortical train stimulation.

Response	Treatment		
	Control	ODQ	ODQ + 7NI
E	38.7% (12/31 cells)	36.0% (9/25 cells)	13.8% (4/29 cells) *
I	35.5% (11/31 cells)	36.0% (9/25 cells)	27.6% (8/29 cells)
NR	25.8% (8/31 cells)	28.0% (7/25 cells)	58.6% (17/29 cells) * ^{&}

Ratios in parentheses indicate the number of cells exhibiting the indicated type of response per number of cells tested.

* $p < 0.05$ as compared to vehicle control group using a Fisher Exact Test.

[&] $p < 0.05$ as compared to ODQ treated group using a Fisher Exact Test. Data were derived from $n = 9-15$ rats per group. Abbreviations: E, excitatory; I, inhibitory; NR, no response.