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Feed-forward excitation of striatal neuron activity by frontal cortical activation of nitric oxide signaling *in vivo*

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Abstract

The gaseous neurotransmitter nitric oxide plays an important role in the modulation of corticostriatal synaptic transmission. This study examined the impact of frontal cortex stimulation on striatal nitric oxide efflux and neuron activity in urethane-anesthetized rats using amperometric microsensor and single-unit extracellular recordings, respectively. Systemic administration of the neuronal nitric oxide synthase inhibitor 7-nitroindazole decreased spontaneous spike activity without affecting activity evoked by single-pulse stimulation of the ipsilateral cortex. Train (30 Hz) stimulation of the contralateral frontal cortex transiently increased nitric oxide efflux in a robust and reproducible manner. Evoked nitric oxide efflux was attenuated by systemic administration of 7-nitroindazole and the non-selective nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester. Train stimulation of the contralateral cortex, in a manner identical to that used to evoke nitric oxide efflux, had variable effects on spike activity assessed during the train stimulation trial, but induced a short-term depression of cortically evoked activity in the first post-train stimulation trial. Interestingly, 7-nitroindazole potently decreased cortically evoked activity recorded during the train stimulation trial. Moreover, the short-term depression of spike activity induced by train stimulation was enhanced following pretreatment with 7-nitroindazole and attenuated after systemic administration of the dopamine D2 receptor antagonist eticlopride. These results demonstrate that robust activation of frontal cortical afferents in the intact animal activates a powerful nitric oxide-mediated feed-forward excitation which partially offsets concurrent D2 receptor-mediated short-term inhibitory influences on striatal neuron activity. Thus, nitric oxide signaling is likely to play an important role in the integration of corticostriatal sensorimotor information in striatal networks.

Introduction

Nitric oxide (NO) is a gaseous neuronal messenger which has been shown to play a variety of important roles in regulating striatal function via its influence on numerous physiological and pathophysiological processes. NO is formed via an NO synthase (NOS)-dependent process which catalyzes the conversion of L-arginine and oxygen into citrulline and NO co-products (Palmer *et al*., 1988). Although three distinct isoforms of NOS are expressed in the nervous system (Alderton *et al*., 2001), neuronal NOS (nNOS) has been shown to play a predominant role in modulating synaptic transmission (Garthwaite & Boulton, 1995). nNOS is bound to postsynaptic density protein PSD-95 which anchors it in a functional complex with the NMDA receptor (Christopherson *et al*., 1999). Thus, NO is generated proximal to the plasma membrane and can diffuse freely out of the cell and modulate neurotransmission via its interaction with downstream signaling molecules (Boehning & Snyder, 2003).

In the striatum, nNOS activity is critical for NO generation and is localized exclusively to medium-sized aspiny GABAergic/somatostatinergic interneurons (Kubota *et al*., 1993; Kharazia *et al*., 1994). NOS interneurons in the dorsal striatum and nucleus accumbens receive asymmetric glutamatergic synaptic contacts from the frontal cortex (Vuillet *et al*., 1989; Salin *et al*., 1990) and ventral subiculum (French *et al*., 2005), respectively. Studies using electrophysiological and molecular techniques both indicate that NOS interneurons are potently activated by stimulation of corticostriatal afferents (Kawaguchi, 1993; Berretta *et al*., 1997). Consistent with the above, corticostriatal glutamatergic transmission is thought to activate striatal nNOS primarily via NMDA receptor and calcium/calmodulin-dependent mechanisms (Marin *et al*., 1992; East *et al*., 1996; Kendrick *et al*., 1996; Morris *et al*., 1997; Iravani *et al*., 1998; Crespi *et al*., 2001; Crespi & Rossetti, 2004; Sammut *et al*., 2007b).

Approximately 90–95% of striatal neurons are medium-sized projection neurons (MSNs), which are distinguished from other striatal cells based on their unique physiological properties and dense spine-laden dendritic processes (for a review, see Bolam *et al*., 2000). Recent studies using both *in vitro* and *in vivo* intracellular recording techniques indicate that nNOS interneurons potently modulate corticostriatal synaptic plasticity and membrane excitability of identified MSNs via the guanylyl cyclase (GC)-cGMP second messenger cascade (Calabresi *et al*., 1999a,b, 2000; Centonze *et al*., 2003; West & Grace, 2004). Intense nitrergic signaling may also function to increase electrotonic coupling (O'Donnell & Grace, 1997) and amplify glutamate-driven oscillations (Sammut *et al*., 2007b) in striatal neurons, both of which may facilitate coherent corticostriatal transmission and synchronize striatal output. The regulation of MSN activity by NOS interneurons is likely to be complex as these cells are

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capable of releasing neuropeptide Y, somatostatin and GABA as cotransmitters (Kawaguchi, 1997). Additionally, studies using extracellular recordings combined with local or systemic drug administration have reported both excitatory (West *et al*., 2002; Liu *et al*., 2005a) and inhibitory (Sardo *et al*., 2002; Di Giovanni *et al*., 2003) effects of NO generators on striatal neuron activity. Together with reports indicating an important role for striatal NO–GC signaling in motor behavior (for a review, see Del Bel *et al*., 2005), these studies suggest that characterization of the complex signaling mechanisms utilized by NOS-containing interneurons will be essential for understanding normal striatal function and pathophysiological conditions such as Parkinson's disease.

The current study was undertaken to examine the impact of tonic and phasic NO signaling on cortically evoked activity and short-term plasticity of putative striatal MSNs. Thus, electrical stimulation techniques were used in conjunction with *in vivo* amperometric microsensor recordings of striatal NO efflux and single-unit extracellular recordings of striatal neuron activity.

Methods

Drugs

Urethane, 7-nitroindazole (7-NI), N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME), eticlopride hydrochloride, and Cremophor EL were purchased from Sigma (St Louis, MO, USA). (+/–)*S*-nitroso-*N*-acetyl-penicillamine (SNAP) used for calibration of NO electrodes was obtained from World Precision Instruments (WPI, Sarasota, FL, USA). All other reagents were of the highest grade commercially available.

Subjects and surgery

Electrochemical and electrophysiological recordings were made from 75 male Sprague–Dawley (Harlan, Indianapolis, IN, USA) rats weighing 250–415 g. Prior to use, animals were housed two per cage under conditions of constant temperature (21–23 °C) and maintained on a 12/12-h 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) and placed in a stereotaxic apparatus (Narashige International USA Inc., East Meadow, NY, USA or David Kopf Instruments, Tujunga, CA, USA) so that the skull was set in a horizontal plane. A solution of lidocaine HCl (2%) and epinephrine (1 : 100 000) (Henry Schein, Melville, NY, USA) was injected into the scalp (s.c.) in a volume of ~0.3 mL and allowed to diffuse for several minutes. Next, an incision (~2–4 cm) was made in the scalp and and burr holes (~2–3 mm in diameter) were drilled in the skull overlying both hemispheres 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 to 2.0 mm anterior from bregma, 2.0–3.5 mm lateral from the midline). The dura mater was resected and the stimulating electrode and microsensor/recording electrode were lowered into the brain using a Narishige or Kopf micromanipulator. All coordinates were determined using a rat brain stereotaxic atlas (Paxinos & Watson, 1986). 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, 2007a,b). Temperature was monitored using a rectal probe and maintained at 37 °C using a heating pad (Vl-20F, Fintronics Inc., Orange, CT, USA).

Electrical stimulation

In NO microsensor experiments, a single bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) was implanted into the frontal cortex (coordinates: 2.7–4.2 mm anterior to bregma, 1.5–2.5 mm lateral to midline, 2.0–4.2 mm ventral to the brain surface) contralateral to the microsensor as previously described (Sammut et al., 2007b). In electrophysiological studies, bipolar stimulating electrodes were implanted either ipsilaterally or bilaterally into the frontal cortices (coordinates were within the same range as above). Single-pulse stimulation was delivered via the electrode implanted ipsilateral to the recording pipette, whereas train stimulation was delivered via the contralateral electrode. Electrical stimuli with durations of 200–500 μs and intensities between 0.25 and 1.25 mA were generated using a stimulator and photoelectric constant current/stimulus isolation unit (S88 stimulator with PSIU6F stimulus isolation unit, Grass Instruments, Quincy, MA, USA) and delivered in single pulses (0.5 Hz) for a duration of 200 s or as stimulus trains [30 Hz, 1000-ms train duration, 2-s intertrain interval (ITI)] for a duration of 50 s. 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. Train stimulation parameters were chosen to approximate the natural spike activity (spikes per burst, intraburst frequency, and bursts per second) of corticostriatal pyramidal neurons (Cowan & Wilson, 1994).

Electrochemical detection of nitric oxide

Striatal NO levels were determined using an NO-selective, amperometric microsensor (amiNO-100, Innovative Instruments, Inc., Tampa, FL, USA or ISO-NOPF200, WPI) as previously described (Liu *et al*., 2005b; Sammut *et al*., 2006, 2007a,b). Briefly, the NO oxidation current (electrode potential of 0.865 V against a built-in Ag/AgCl reference electrode) was amplified and recorded using a commercially available system (Apollo 4000, WPI). Prior to each experiment, the NO microsensor was calibrated in a temperature controlled chamber (Diamond General, Ann Arbor, MI, USA) using known concentrations of the NO-generating compound SNAP (Ohta *et al*., 1997; Zhang, 2004; Liu *et al*., 2005b; Sammut *et al*., 2006, 2007a,b). Calibration plots were constructed prior to each experiment in order to determine the sensitivity and integrity of the electrode and confirm that the NO oxidation current exhibited a linear response to NO concentrations ranging from 0.6 to 48 nM. The selectivity of the NO microsensor for NO was also tested *in vitro* using known concentrations of other electrochemically active compounds found at nanomolar concentrations in the dorsal striatum and drugs (≤ 1 μM) utilized in the current study (Sammut *et al*., 2006, 2007a,b).

Extracellular recordings

Extracellular recording microelectrodes were manufactured from 2.0-mm outer-diameter borosilicate glass capillary tubing (WPI, New York, NY) using a vertical micropipette puller (model PE-21, Narishige) as previously described (West & Grace, 2000; Floresco *et al*., 2003). Briefly, electrodes were broken back against a glass rod under microscopic control to approximately 1 μm tip diameter and filled with 2 M NaCl. Microelectrode resistance was measured *in vivo*

at 1000 Hz with a current of 10 nA using an FHC Xcell 3+ microelectrode amplifier/impedance check (Frederick Haer Company (FHC), Bowdoinham, ME, USA) and ranged from 10 to 15 MΩ. Previous studies have shown that the majority of striatal neurons are silent (Mallet *et al*., 2005). Thus, in order to isolate single units, extracellular microelectrodes were lowered incrementally through the dorsal striatum using a micromanipulator (MO-8, Narashige) while single-pulse electrical stimuli (0.5 Hz, 0.5 ms, 0.25–1.25 mA) were applied to the cortex (see above). Once a cell was detected, the position of the recording electrode was adjusted to maximize the spike signal to background noise ratio (≥ 4 : 1). In within-subjects studies, the impact of varying the intensity of electrical stimuli within a given range of current (0.4–0.8 mA) on evoked spike activity was assessed over 50 stimuli per trial. In between-subjects studies, stimulation currents were titrated to an intensity (280–1250 μA) which reliably evoked spike activity approximately 50% of the time (e.g. ∼50 spikes in response to 100 stimulus pulses delivered at 0.5 Hz). Extracellular electrode potentials were passed through a high-input impedance amplifier/window discriminator (Fintronics WDR 420, or FHC Xcell 3+ microelectrode amplifier) connected to a Grass AM-10 audio monitor and displayed on a BK Precision 2120B dual-trace (30 mHz) oscilloscope. Single-unit activity was filtered (low-pass filter at 8 kHz and high-pass filter at 400 Hz), digitized (20 kHz) and recorded using an Intel™-based microcomputer with a data acquisition board interface (DAP-5200a, Microstar Laboratories, Bellevue, WA, USA). Detailed analysis of all spontaneous and evoked spikes was performed off-line using custom (Neuroscope) software applications (Brian Lowry, Pittsburgh, PA, USA).

Drug preparation/administration and experimental protocol

Urethane and eticlopride were dissolved in physiological saline (0.9%). 7-NI and L-NAME were dissolved in vehicle consisting of 10% Cremophor EL in saline (0.9%) solution (Eblen *et al*., 1996; Sammut *et al*., 2006, 2007b). Effective doses of 7-NI (50 mg/kg, i.p.), L-NAME (100 mg/kg, i.p.) and eticlopride (100 μg/kg, i.p.) were derived from the range previously reported in the literature (Ohta *et al*., 1994; Eblen *et al*., 1996; Kalisch *et al*., 1996; Ayers *et al*., 1997; Adachi *et al*., 2000; Hirabayashi *et al*., 2001; Sammut *et al*., 2006, 2007a). In NO microsensor experiments, the response to frontal cortical stimulation was assessed multiple times prior to drug or vehicle administration, with stimulations separated by 15–20 min from each other in order to obtain at least two consecutive responses that did not vary by > 20% (Sammut *et al*., 2006, 2007a,b). The average of these measures was considered as the baseline response to which responses following drug or vehicle administration were compared. Previous time-course studies of the impact of NOS inhibitors on NO synthesis in the striatum have shown that systemic (i.p.) injections of 7-NI and L-NAME reduced NOS activity to ∼50–60% of control levels (Adachi *et al*., 2000). Moreover, the effects of 7-NI and L-NAME developed during the first 30–80 min post-injection and were sustained for > 180 min post-injection (Adachi *et al*., 2000). Thus, in the current study, the impact of vehicle, 7-NI and L-NAME on cortically evoked NO efflux was measured 20–120 min post-injection.

In single-unit recording studies, vehicle or 7-NI (50 mg/kg, i.p.) was administered using either a within- or a between-subjects design. Eticlopride was administered using a within- subjects design. In within-subjects studies using 7-NI, after isolating a striatal neuron and recording spontaneous (non-evoked) firing activity (∼2–5 min), the effects of separate trials (50 pulses each) of single-pulse cortical stimulation (0.4, 0.6, and 0.8 mA stimulation intensities) were recorded prior to vehicle or drug administration. Next, vehicle or 7-NI was administered and spontaneous firing and the effects of single-pulse electrical stimulation were reassessed approximately 20–30 min later as described above. In between-subjects studies using 7-NI, animals were injected with either vehicle (10% Cremophor EL in saline) or 7-NI (in vehicle) at least 25 min prior to initiation of the recording session. After isolating a cell as described above, stimulation currents were adjusted to approximately 50% maximal responding to stimulation delivered at 0.5 Hz. A pre-train stimulation baseline trial consisting of 100 individual single-pulse stimulations delivered over 200 s 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 (30 Hz, 1000 ms train duration, 2 s ITI for a duration of 50 s). In order to examine the impact of train stimulation on spike probability during the train stimulation trial, single-pulse stimulation was delivered concurrently to the ipsilateral cortex via a second stimulation channel which was triggered by channel 1 (train) stimulation but delayed ∼500 ms from the end of the stimulus train (see Figs 1 and 5). Immediately following the train stimulation trial, three additional post-train stimulation trials (200 s each) were performed in a manner which was identical to the pre-train stimulation trial (100 single-pulse trials each, no train stimulation). Three post-train trials were chosen based on the observation that train-induced changes in spike probability in control animals were usually observed to return to prestimulation levels during the second or third post-train stimulation trials, recorded approximately 200–600 s after completion of the train stimulation trial. Within-subjects studies using eticlopride followed this same experimental protocol, except the responsiveness of striatal neurons to the cortical train stimulation protocol was re-tested at least 20 min after drug administration. As these experiments were designed to determine whether dopamine D2 receptor activation mediates the inhibition of cortically evoked spike activity induced by cortical train stimulation, only neurons exhibiting an inhibitory response were selected for further experimentation. Additionally, all striatal neurons included in the data set exhibited stable electrophysiological properties (see above) for a minimum of 20 min following drug administration and throughout the duration of the post-injection stimulation protocol.

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, USA). Brains were removed and post-fixed in 10% formalin/PB for at least 3 days. Next, brains were immersed in PBS/sucrose solution (30%) and stored in a refrigerator 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 electrode/NO microsensor sites (Sammut *et al*., 2007b).

Data analysis

In amperometric recording experiments, the NO oxidation current (pA) was allowed to stabilize for at least 150 s prior to stimulation. The NO oxidation current recorded (50 Hz sampling frequency) over the last 30 s of the pre-stimulation period was then averaged

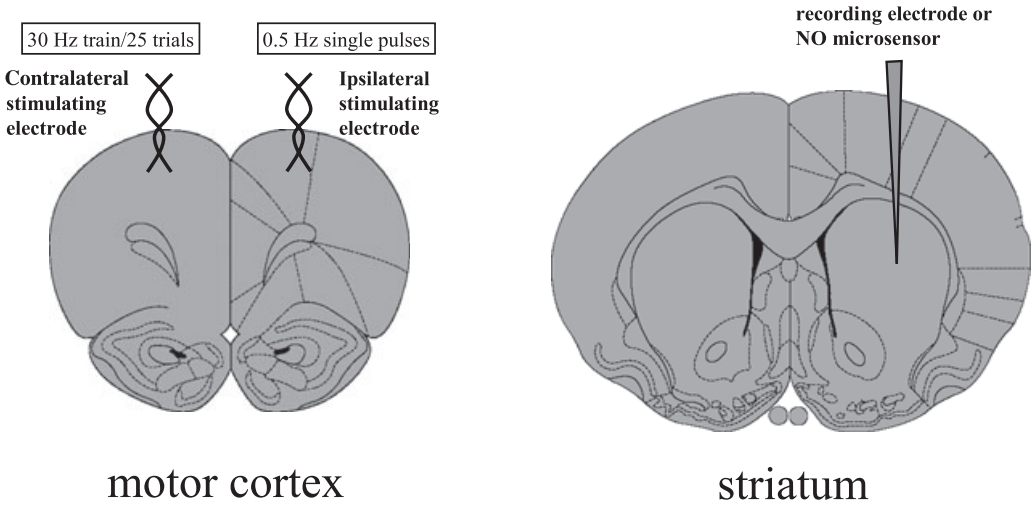

FIG. 1. Overview of stimulating and recording protocols. Stimulating electrodes were implanted bilaterally into the frontal cortex (left) as described in the Methods section. In all electrophysiological studies, an extracellular recording electrode was implanted into the striatum (right) and single-unit activity evoked by single-pulse stimulation of the ipsilateral cortex was recorded prior to recording spontaneous firing activity. In studies involving NO microsensor measurements, NO efflux evoked via train stimulation of the contralateral motor cortex was recorded in the dorsal striatum. In studies examining the impact of train stimulation on cortically evoked spike activity, train stimulation was delivered to the contralateral motor cortex, whereas single-pulse stimulation was delivered to the ipsilateral motor cortex to evoke single-unit spike activity. The probability of evoking spike activity during single-pulse stimulation of the ipsilateral cortex was recorded prior to, during and after train stimulation of the contralateral cortex (diagrams are derived from the atlas of Paxinos & Watson, 1986).

using Apollo 4000 software applications (WPI) and subtracted from the mean NO oxidation current recorded during the last 30 s of the stimulation period (Liu *et al*., 2005b; Sammut *et al*., 2006, 2007a,b). The mean stimulation-evoked concentration of NO (nM) is derived from *in vitro* calibration curves (Sammut *et al*., 2006, 2007a,b). In single-unit recording studies, firing rate histograms were constructed (1.0-ms bins of 2-min epochs) from data recorded prior to and following (20–30 min) 7-NI administration. Peri-stimulus time histograms (PSTHs) were constructed (1.0-ms bins) for each stimulus 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 (Floresco *et al*., 2001). Single-unit and group data were summarized using population PSTHs and/or spike probability plots as indicated. Excitatory (E) responses observed during train stimulation were operationally defined as an increase in spike probability of >2 SD above the pretrain stimulation mean. Inhibitory (I) responses observed during train stimulation were operationally defined as a decrease in spike probability of >2 SD below the pretrain stimulation mean. This ± 2 SD range was selected after studying the spike probability changes of five single units recorded following vehicle injection during a 10–40-min post-injection period (see Tseng *et al*., 2000). Similar criteria were used in the assessment of stimulation-induced changes in evoked spike activity in a previous study (Jodo *et al*., 2005). The statistical significance of drug- and stimulation-induced changes in NO oxidation current and spike activity was determined using either a paired *t*-test/Wilcoxon signed rank test, Fisher exact test or one-/two-way analysis of variance (ANOVA) with repeated measures (RM) as indicated (Sigma Stat, Jandel). Also, either a Bonferroni or Tukey *post-hoc* test was used as indicated to determine which group(s) contributed to overall differences seen with ANOVA. Data pertaining to NO efflux are graphically represented as the mean ± SEM percentage of predrug/vehicle control. Data from single-unit recordings are graphically represented as the mean ± SEM, spikes per bin, firing rate, spike probability, spike latency and standard deviation of spike latency.

Results

Stimulating electrode, microsensor and recording electrode placements

All identified stimulating electrode tips were confirmed to lie in the frontal cortex between 2.7 and 4.2 mm anterior to bregma, 0.8 and 2.5 mm lateral to the midline, and 2.0 and 5.5 mm ventral to the surface of the skull (Paxinos & Watson, 1986; Fig. 1). Identified placements for NO microsensors and extracellular recording electrodes implanted into the striatum were verified to lie between 0.26 mm posterior and 1.6 mm anterior to bregma, 1.7 and 4.0 mm lateral to the midline, and 3.4 and 7.8 mm ventral to the dural surface (Paxinos & Watson, 1986; Fig. 1).

Modulation of spontaneous and cortically evoked spike activity by tonic NO

To assess the impact of endogenous NO tone on spontaneous and cortically evoked spike activity, striatal neurons were recorded before and after (~20–30 min) systemic administration of vehicle (10% Cremophor EL in 0.9% saline) or the selective nNOS inhibitor 7-NI (50 mg/kg, i.p.). It is estimated that 90–95% of striatal neurons are projection neurons (Wilson, 2004). 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] were not recorded in this study (Wilson *et al*., 1990; Kawaguchi, 1993; Plenz & Kitai, 1998; Koós & Tepper, 1999; Mallet *et al*., 2005, 2006). Given the above, it is highly likely that the vast majority of single units recorded in this study were MSNs. In within-subjects studies, successful recordings of evoked activity before and after vehicle or 7-NI administration were obtained for $n = 5$ and $n = 15$ cells/rats, respectively. After isolating a single unit using single-pulse stimulation of the ipsilateral cortex (see Methods), 2–5 min of spontaneous firing activity was recorded

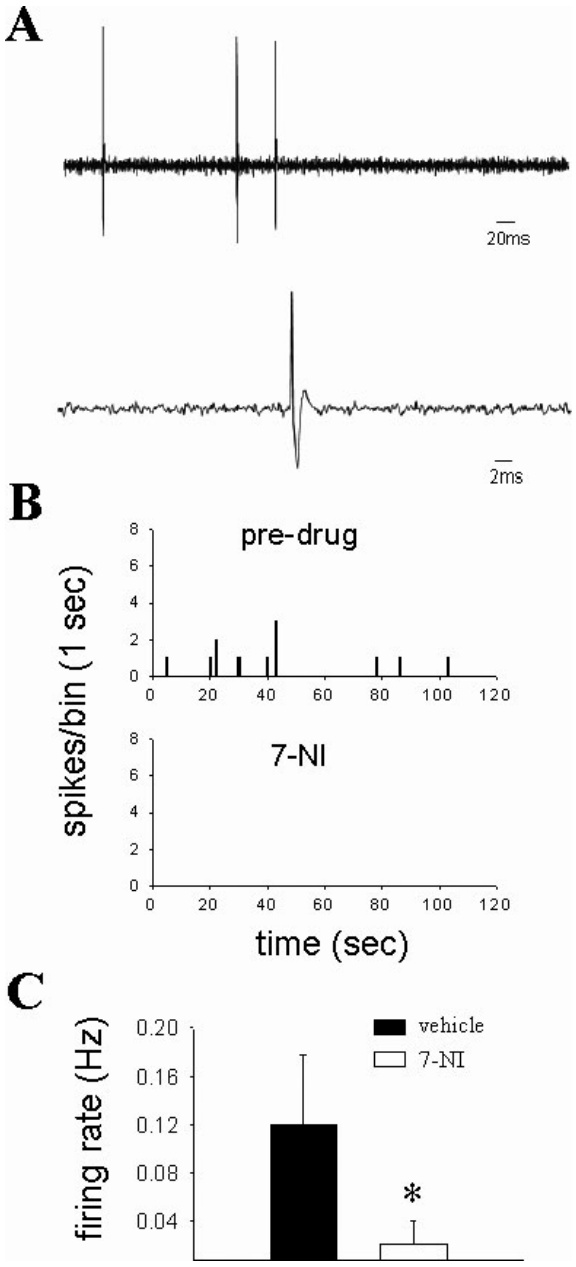

FIG. 2. Inhibition of nNOS activity decreases the spontaneous activity of striatal neurons isolated via electrical stimulation of the frontal cortex. (A) Top: representative trace of spontaneous spike activity of a striatal neuron recorded prior to systemic administration of 7-NI (50 mg/kg, i.p.). Bottom: magnification of the first spike shown in the above trace. (B) Representative firing rate histograms showing the spontaneous activity (120 s) of a single striatal neuron (same cell as in A) before (top) and after (bottom) 7-NI administration. As noted above, the majority of neurons exhibiting spontaneous activity under basal conditions were quiescent following 7-NI administration (4/7 spontaneously active cells). Importantly, following 7-NI administration spike activity was consistently evoked in all neurons by cortical stimulation. (C) 7-NI administration decreased the mean ± SEM firing rate of single units previously isolated via electrical stimulation of the frontal cortex (*$P < 0.05$; Wilcoxon rank test, $n = 14$ cells/rats).

(Fig. 2). Vehicle administration (10–40 min) did not induce significant changes in spontaneous firing activity (data not shown). As shown in Fig. 2B and C, 7-NI administration significantly depressed spontaneous firing activity ($P < 0.05$, Wilcoxon signed rank test) without affecting the proportion of spontaneously active cells within the group (basal firing rates were collected for 14 total cells/rats; pre-7-NI: seven spontaneously firing, seven quiescent; post-7-NI: three spontaneously firing, eleven quiescent; $P > 0.05$, Fisher exact test).

To examine the potential effects of tonic NO on spike activity evoked by electrical stimulation of frontal cortical afferents, multiple stimulation trials consisting of 50 single pulses (0.5 Hz, 200–500 μs, 400–800 μA) were delivered to the ipsilateral cortex in separate trials both before and 20–30 min after vehicle or 7-NI administration (Fig. 3A and B). Vehicle administration did not affect the mean ± SEM probability of evoking short-latency spike activity during single pulse stimulation of the ipsilateral frontal cortex ($P > 0.05$; two-way repeated-measures (RM) ANOVA with two factor repetition, data not shown). Short-latency spike activity evoked during repeated single-pulse stimulation of the ipsilateral frontal cortex was dependent on the level of current intensity, both prior to and following 7-NI administration (Fig. 3C; $F_{2,28} = 38.73$, $P < 0.001$; two-way RM ANOVA with two factor repetition and Tukey *post-hoc* test, $P < 0.005$ at all levels of current intensity). No significant differences in mean ± SEM spike probability or interactions between drug and current intensity were observed between measures taken prior to and following 7-NI administration (Fig. 3C; $P > 0.05$; two-way RM ANOVA with two factor repetition). The average onset latency of evoked spikes and standard deviation of spike latency was calculated from recordings of unit activity during stimulation trials performed using the highest tested current intensity (800 μA), prior to and following vehicle or 7-NI administration (20–30 min). Vehicle and 7-NI administration did not affect the mean ± SEM onset latency of evoked spikes (Fig. 3C; $P > 0.05$; two-way RM ANOVA with two factor repetition) or standard deviation of spike latency (Fig. 3C, pre-vehicle: 1.29 ± 0.46 ms; post-vehicle: 1.40 ± 0.39 ms; pre-7-NI: 0.94 ± 0.19 ms; post-7-NI: 1.21 ± 0.20 ms).

Impact of electrical train stimulation of the contralateral frontal cortex on striatal NO efflux: effects of NOS inhibitor administration

Afferents from the contralateral frontal cortex were activated using electrical stimuli delivered for 50 s as trains (25) of high-frequency stimulation (30 Hz, 1000-ms train duration, 2-s ITI, see Methods). The duration of the stimulation period and the stimulation pattern were designed to approximate the natural phasic firing activity of cortical pyramidal neurons observed in conscious behaving animals (Steriade, 2001). To determine the potential role of NOS activation in the effects of electrical train stimulation of the contralateral cortex on striatal NO efflux, train stimulation of the contralateral cortex was carried out as described above (see Methods; Fig. 1) prior to and following (20–120 min) systemic administration of either vehicle or drug. Experiments were performed using L-NAME, a non-selective inhibitor of all known NOS isoforms (100 mg/kg, i.p.), or the selective nNOS inhibitor 7-NI (50 mg/kg, i.p.). Vehicle administration did not affect NO efflux evoked by electrical train stimulation of the contralateral frontal cortex (Fig. 4A; $P > 0.05$, one-way RM ANOVA on measures taken 20 min prior to and 20–120 min after vehicle injection). NO efflux observed during cortical train stimulation was attenuated following systemic administration of either 7-NI (Fig. 4; $F_{6,29} = 8.23$, $P < 0.001$, one-way RM ANOVA and Bonferroni *t*-test, $P < 0.01$ at 100–120 min post 7-NI) or L-NAME (Fig. 4C; $F_{6,33} = 8.10$, $P < 0.001$, one-way RM ANOVA and Bonferroni *t*-test, $P < 0.005$ at 80–120 min post L-NAME).

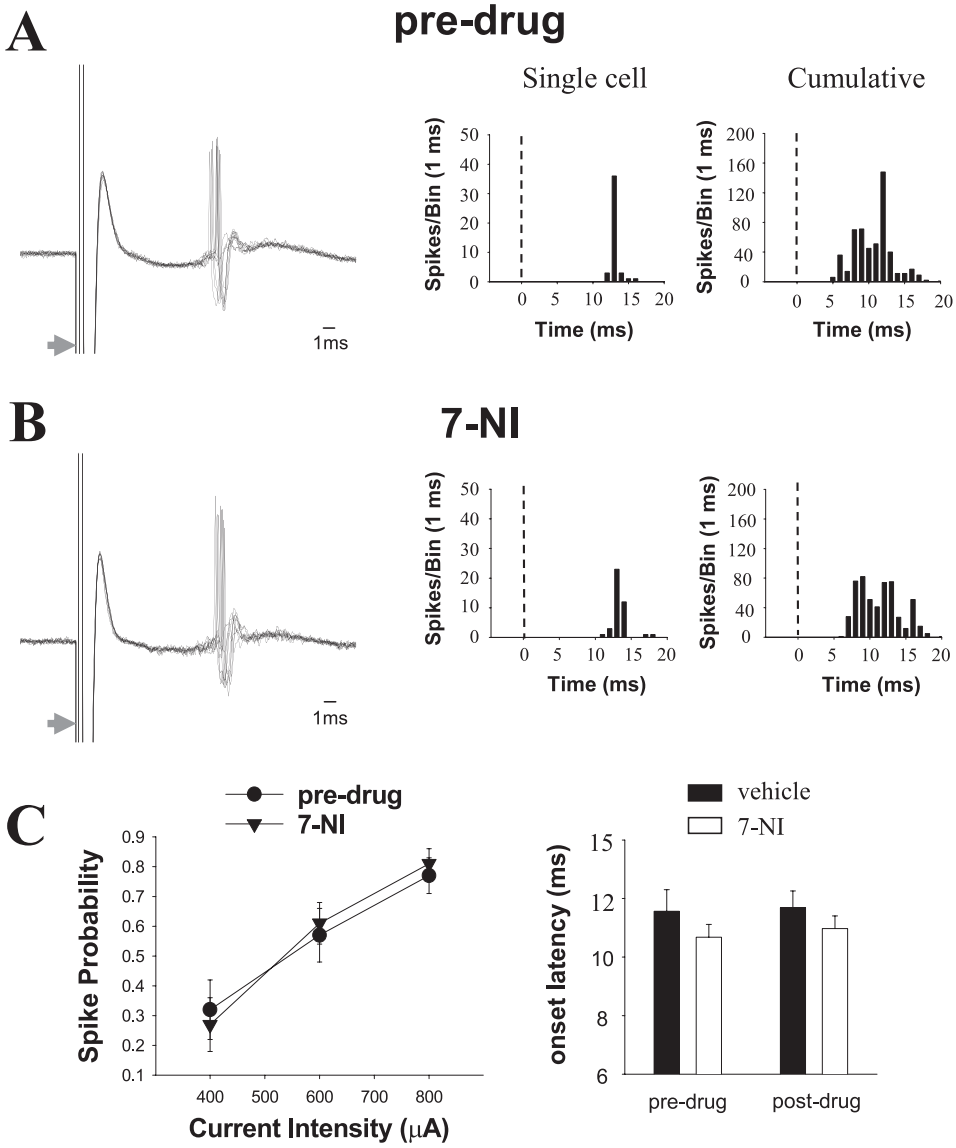

FIG. 3. Effects of nNOS inhibition on single-unit and population activity evoked via electrical stimulation of the frontal cortex. (A) Left: representative traces of cortically evoked spike responses of a single-unit (overlayed records of the last ten stimulations per trial) recorded prior to 7-NI administration. Middle: representative PSTH showing the response of the same cell to cortical stimulation before 7-NI administration. Right: cumulative PSTH depicting the population response of all 15 striatal neurons to cortical stimulation before 7-NI administration. (B) Left: representative traces of cortically evoked spike responses (overlayed records of the last ten stimulations per trial) of the same single unit shown above recorded 20–30 min after 7-NI administration (50 mg/kg, i.p.). Middle: representative PSTH showing the response of the same single unit shown above to cortical stimulation delivered 20–30 min after 7-NI administration. Right: cumulative PSTH depicting the population response of all 15 striatal neurons to cortical stimulation 20–30 min after 7-NI administration. All histograms shown in A and B were generated from 50 stimulation trials. Arrows indicate the location of the stimulus artifact. Dashed vertical lines indicate the onset of single-pulse electrical stimulation (0.5 Hz, 200–500 μs, 800 μA) of the frontal cortex. (C) Left: cortical stimulation induced a stimulus intensity-dependent increase in spike probability prior to and following 7-NI administration ($P < 0.05$; two-way RM ANOVA, $n = 15$ cells/rats). However, 7-NI administration did not affect the probability of eliciting spike activity during cortical stimulation ($P > 0.05$; two-way RM ANOVA, $n = 15$ cells/rats). Right: vehicle and 7-NI administration did not affect the mean ± SEM onset latency of single spike responses to single-pulse electrical stimulation of the frontal cortex ($P > 0.05$; two-way RM ANOVA, $n = 15$ cells/rats).

Impact of electrical train stimulation of the contralateral frontal cortex on cortically evoked activity: effects of NOS inhibitor administration

To examine the effects of NO generated in a phasic manner on cortically evoked spike activity, train stimulation of the contralateral cortex was performed in a manner identical to that used to induce NO efflux in the above neurochemical studies. In these between-subjects studies, animals were injected with either vehicle or 7-NI at least 25 min prior to initiation of the recording session. The majority of neurons in both groups were recorded >80 min post-injection, a time range that corresponded with the maximal inhibition of evoked NO efflux observed following NOS inhibitor administration (see Fig. 4). No significant differences in the mean ± SEM time of recording (post-injection) existed between groups ($P > 0.05$; *t*-test, vehicle: 111.1 ± 9.6 min; 7-NI: 89.7 ± 8.3 min). After isolating a cell, the current intensity of stimulation pulses delivered to the ipsilateral cortex was adjusted to approximately 50% maximal responding and pretrain stimulation baseline trials (100 pulses) were recorded (Figs 5 and 6). Consistent with the within-subjects studies, no significant differences in the mean ± SEM minimal stimulating current needed to reach a response probability of approximately 50% were observed between groups ($P > 0.05$; *t*-test, vehicle: 869 ± 46 μA; 7-NI: 811 ± 60 μA).

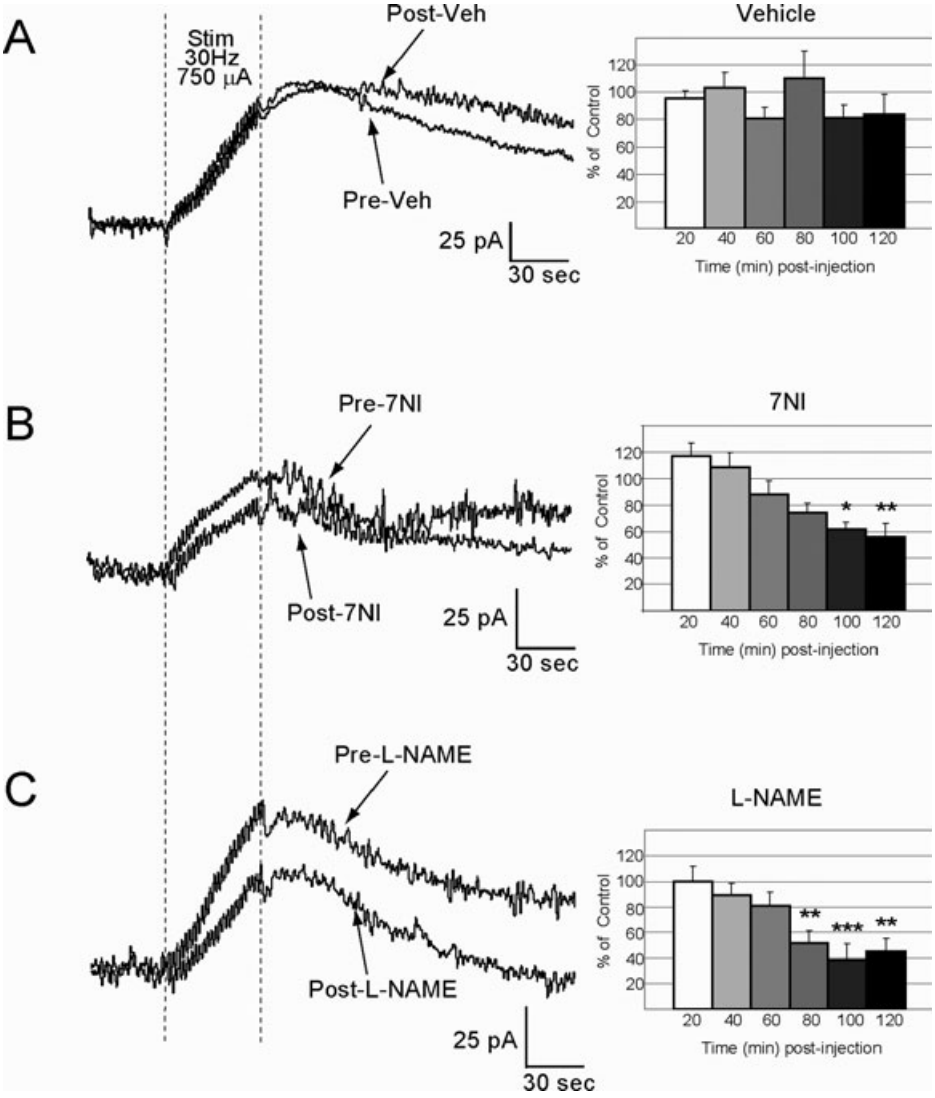

FIG. 4. Impact of train stimulation of the contralateral frontal cortex on striatal NO efflux *in vivo*: effects of systemic NOS inhibitor administration. (A) Left: representative recordings showing the effects of train stimulation (30 Hz, 1000-ms train duration, 2-s ITI, 750 μA, 0.5 ms, 25 trains) on NO efflux evoked prior to and following (120 min) systemic administration of vehicle (Veh). Right: vehicle administration did not affect cortically evoked NO efflux ($P > 0.05$, one-way RM ANOVA, $n = 6$ rats; mean ± SEM cortically evoked NO concentrations prior to vehicle administration were 4.5 ± 1.8 nM). (B) Left: representative recordings showing the effects of train stimulation (30 Hz, 1000-ms train duration, 2-s ITI, 750 μA, 0.5 ms, 25 trains) on NO efflux evoked prior to and following (100 min) systemic administration of 7-NI (50 mg/kg). Right: striatal NO efflux evoked during cortical stimulation was significantly reduced 100–120 min after 7-NI administration (*$P < 0.05$, **$P < 0.005$, one-way RM ANOVA and Bonferroni *t*-test, $n = 7$ rats, mean ± SEM crtically evoked NO concentrations prior to 7-NI administration were 4.3 ± 0.5 nM). (C) Left: representative recordings showing the effects of train stimulation (30 Hz, 1000-ms train duration, 2-s ITI, 750 μA, 0.5 ms, 25 trains) on NO efflux evoked prior to and following (120 min) systemic administration of L-NAME (100 mg/kg). Right: striatal NO efflux evoked during cortical stimulation was significantly reduced 80–120 min after L-NAME administration (**$P < 0.005$, ***$P < 0.001$, one-way RM ANOVA and Bonferroni *t*-test, $n = 7$ rats, mean ± SEM cortically evoked NO concentrations prior to L-NAME administration were 3.7 ± 0.5 nM). Dashed vertical lines indicate the onset and termination of electrical stimulation.

As shown in Fig. 6 and Table 1, in vehicle-treated animals train stimulation delivered to the contralateral frontal cortex (30 Hz, 1000-ms train duration, 2-s ITI, 25 trains) had variable effects on activity evoked via single-pulse stimulation of the ipsilateral cortex. The most frequently observed response recorded during train stimulation of the contralateral frontal cortex in both vehicle- and 7-NI-treated rats was an inhibition (I response) of spike activity evoked by single-pulse stimulation of the ipsilateral cortex (Figs 5 and 6, left, Table 1). A smaller proportion of neurons recorded in vehicle-treated rats responded to train stimulation of the contralateral frontal cortex with a transient increase (E response) in cortically evoked firing (Fig. 6, right, Table 1). Between-group comparisons of the frequency of various responses revealed a significant increase in the I response type, and an abolition of the E response type, in 7-NI-treated animals (Table 1; Fisher exact test, $P < 0.05$).

Initial between-group comparisons of spike activity evoked during stimulation of the ipsilateral cortex were performed on data sets which included every cell in each group regardless of response classification [I, E, or no response (NR)]. Comparisons of measures assessed prior to train stimulation (pre-train), during train stimulation (train) and following train stimulation (post 1–3) revealed multiple significant overall effects of train stimulation of the contralateral cortex across time (Fig. 7, top; $F_{4,216} = 15.690$, $P < 0.001$; two-way RM ANOVA with one factor repetition, $P < 0.05$ in all cases, Tukey *post-hoc* test),

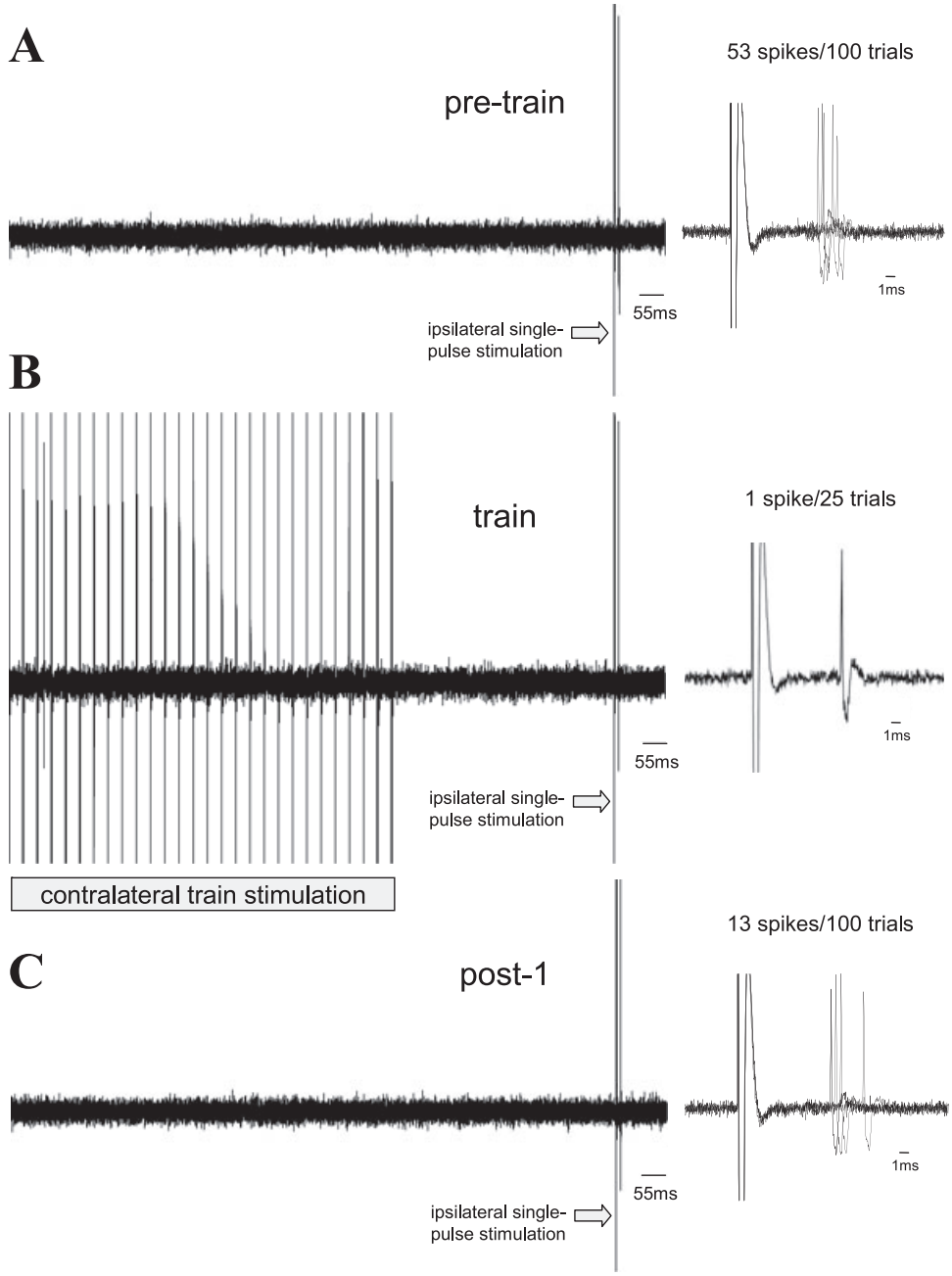

FIG. 5. Modulation of striatal neuron activity during train stimulation of the frontal cortex. (A) Left: representative trace of spike activity evoked by single-pulse stimulation of the ipsilateral frontal cortex in the absence of high-frequency train stimulation (pretrain). Right: overlayed records of ten consecutive stimulations. (B) Left: example of a cortically evoked response elicited by single-pulse stimulation of the ipsilateral frontal cortex delivered ~500 ms after the high-frequency train stimulation (30 Hz, 1000-ms train duration, 2-s ITI, 500 μA, 0.5 ms) of the contralateral frontal cortex (train). Right: magnification of the cortically evoked spike shown on the left. Note that this was the only cortically evoked response observed over 25 train stimulation trials. (C) Left: representative trace of spike activity evoked by single-pulse stimulation of the ipsilateral frontal cortex recorded in the first post-train stimulation trial (post 1). Right: overlayed records of ten consecutive stimulations. Note that during the post 1 stimulation trial (0–200 s after cessation of train stimulation), the neuron begins to recover from the train-induced suppression of cortically evoked activity. All recordings are derived from a single neuron from a vehicle-treated animal. Arrows indicate the location of the artifact from single-pulse electrical stimulation of the ipsilateral frontal cortex.

as well as 7-NI treatment (Fig. 7, top; $F_{1,216} = 5.616$, $P < 0.05$; two-way RM ANOVA with one factor repetition, $P < 0.05$ in all cases, Tukey *post-hoc* test) on cortically evoked activity. The effect of 7-NI treatment also depended on time in relation to the train stimulation trial. Thus, there was a significant interaction between 7-NI administration and time post-train ($F_{4,216} = 4.211$, $P < 0.005$; two-way RM ANOVA with one factor repetition). Interestingly, in vehicle-treated animals train stimulation of the contralateral cortex induced a short-term depression of cortically evoked activity in the first post-train stimulation trial (Fig. 7, top, $P < 0.05$) but did not alter subsequent post-stimulation responses (Fig. 7, top, $P > 0.05$). Moreover, 7-NI administration potently decreased cortically evoked activity during train stimulation of the contralateral cortex (Fig. 7, top, $P < 0.001$) and in the first post-train stimulation trial (Fig. 7, top, $P < 0.001$), but

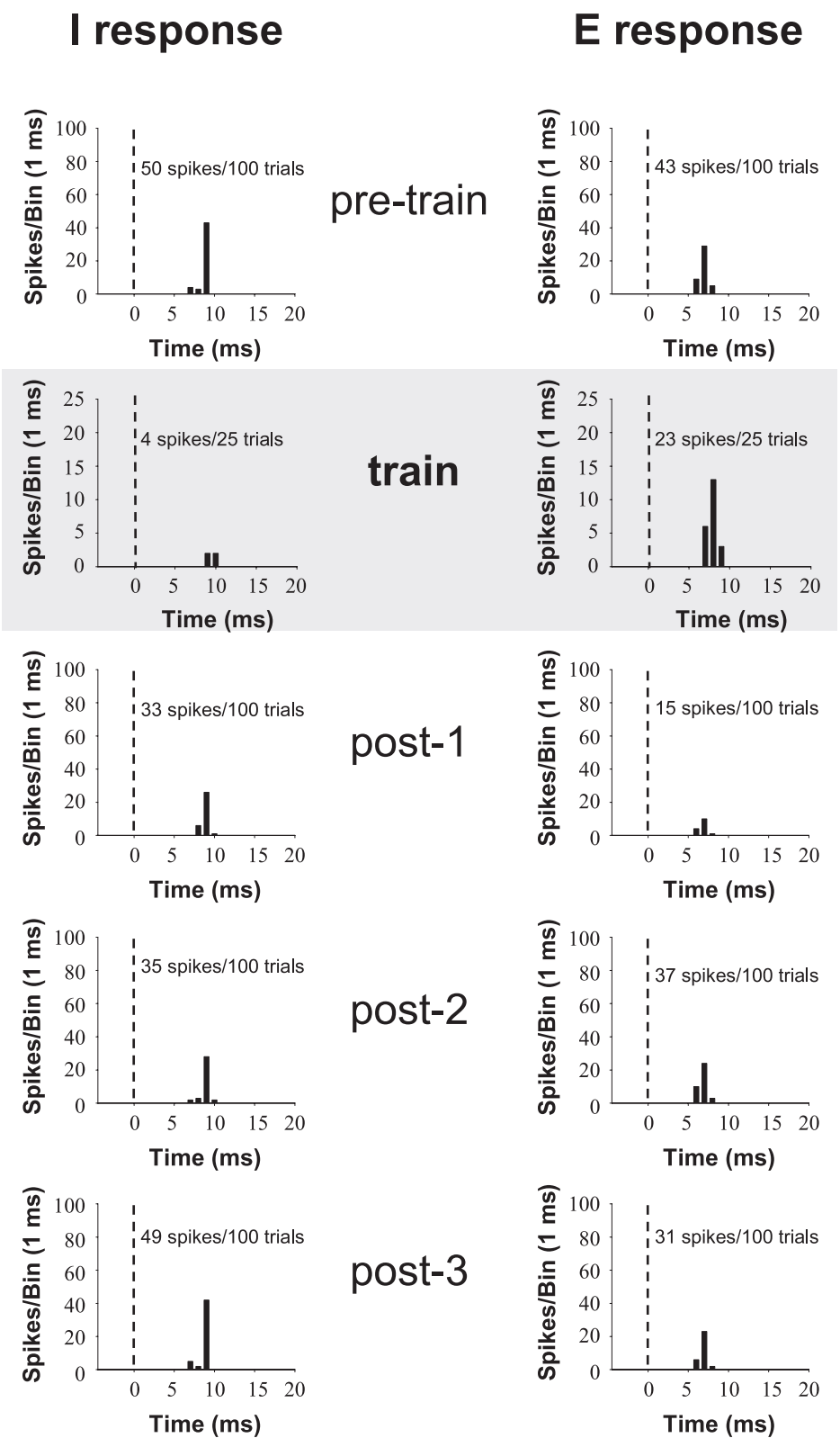

FIG. 6. Inhibitory and facilitatory effects of cortical train stimulation on cortically evoked spike activity. Left: representative PSTHs showing the inhibitory (I) response of a single striatal neuron to cortical stimulation before (pre-train), during (train) and after (post 1–3) train stimulation of the contralateral cortex. Each histogram was generated from 25–100 stimulation trials (50–200 s each) as indicated. Right: representative PSTHs showing the excitatory (E) response of a single striatal neuron to cortical stimulation before (pre-train), during (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. All recordings of I and E responses are derived from single neurons from vehicle-treated animals. Dashed vertical lines indicate the onset of single-pulse electrical stimulation.

TABLE 1. Effects of neuronal NOS inhibition on the observed frequency of inhibitory and excitatory responses of striatal cells during cortical train stimulation.

	Numbers of cells	
Response	Vehicle	7-nitroindazole
Inhibitory (I)	12/29 (41.4%)	21/29 (72.4%)*
Excitatory (E)	6/29 (20.7%)	0/29 (0.0%)*
Non-responsive (NR)	11/29 (37.9%)	8/29 (27.6%)

Ratios indicate the number of cells exhibiting the indicated type of response per number of cells tested. *P<0.05 as compared with vehicle control group using a Fisher exact test. Data were derived from n = 11–13 rats per group.

did not alter subsequent post-stimulation responses. No significant differences in average onset latency of evoked spikes or standard deviation of spike latency were observed between vehicle- and 7-NI-treated groups (Fig. 7, middle, bottom; $P > 0.05$; two-way RM ANOVA with one factor repetition). Lastly, train stimulation was observed to decrease the standard deviation of spike latency of cortically evoked spikes to a similar degree in both vehicle- and 7-NI-treated animals (Fig. 7, bottom, $F_{4,201} = 2.936$, $P < 0.05$; two-way RM ANOVA with one factor repetition).

To determine the impact of 7-NI administration on the magnitude of inhibition observed during and immediately following train stimulation of the contralateral cortex, between-group comparisons of spike activity evoked via single-pulse stimulation of the ipsilateral cortex were performed on neurons exhibiting an I response (Fig. 8). Comparisons of spike probability assessed prior to train stimulation (pre-train), during train stimulation (train) and following train stimulation (post 1–3) revealed multiple significant overall effects of time post-train stimulation (Fig. 8, $F_{4,117} = 51.3$, $P < 0.001$; two-way RM ANOVA with one factor repetition, $P < 0.05$ in all cases, Tukey *post-hoc* test). As described above for the entire data set, the effect of 7-NI treatment on neurons responding to train stimulation with an I

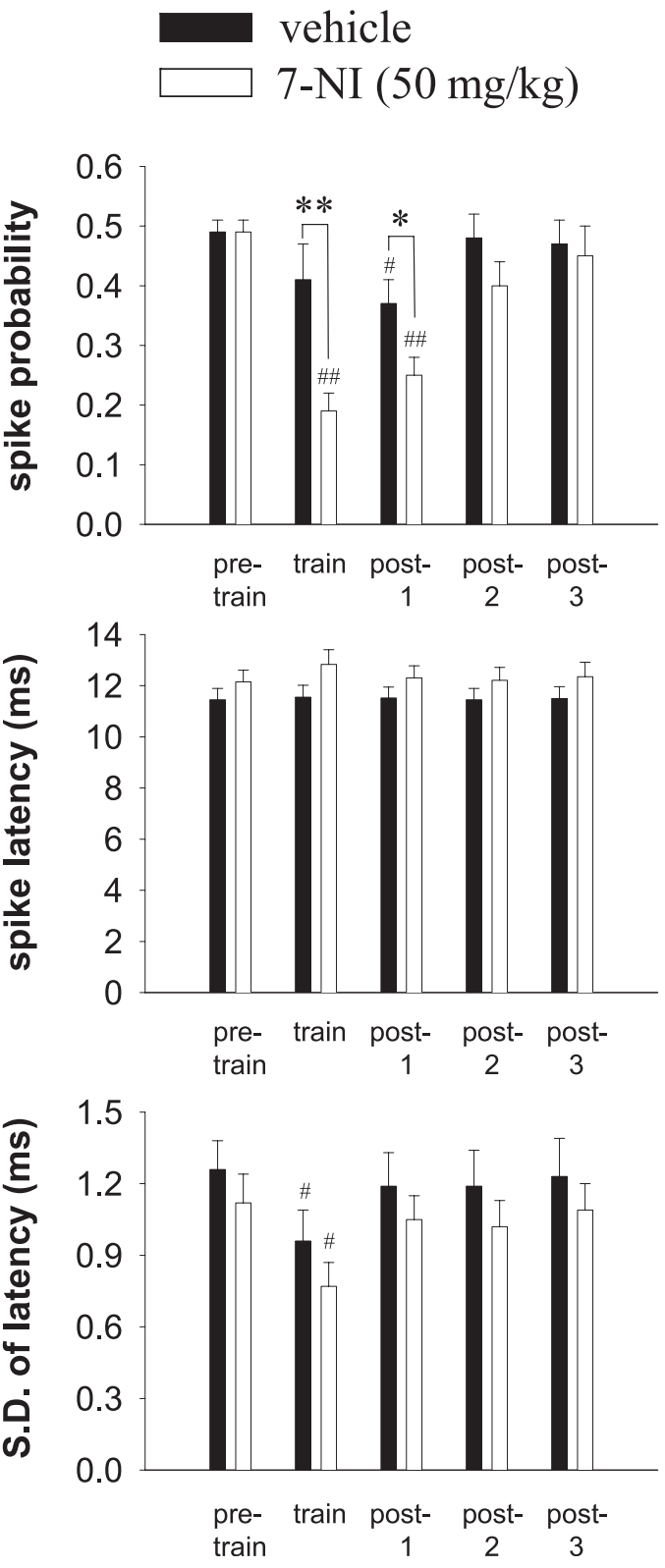

FIG. 7. Inhibition of nNOS activity decreases cortically evoked activity recorded during train stimulation of the cortex and enhances short-term depression of spike activity. Top: within- and between-group comparisons of cortically evoked spike activity measured prior to train stimulation (pre-train), during train stimulation (train) and following train stimulation (post 1–3) in vehicle- and 7-NI-treated rats revealed significant differences resulting from both train stimulation and 7-NI treatment ($P < 0.05$; two-way RM ANOVA with one factor repetition). In vehicle-treated control animals, train stimulation of the contralateral cortex in a manner identical to that used to evoke NO efflux had variable effects on cortically evoked spike activity assessed during the train stimulation trial ($P > 0.05$; two-way RM ANOVA with one factor repetition), but significantly depressed evoked spike activity in the first post-train stimulation trial ($^{\#}P < 0.05$, two-way RM ANOVA with Tukey *post-hoc* test). Pretreatment with 7-NI (50 mg/kg, i.p.) potently decreased cortically evoked spike activity during train stimulation of the cortex ($^{\#\#}P < 0.001$, two-way RM ANOVA) and in the first post-train stimulation trial ($^{\#\#}P < 0.001$, two-way RM ANOVA with Tukey *post-hoc* test). Vehicle and 7-NI administration did not alter subsequent post-stimulation responses (post-2, post-3) to single-pulse stimulation of the cortex ($P > 0.05$; two-way RM ANOVA with one factor repetition). Between-group comparisons revealed that 7-NI-treated animals exhibited a strong suppression of cortically evoked spike activity during the train stimulation trial (**$P < 0.001$, two-way RM ANOVA with Tukey *post-hoc* test), and in the first post-train stimulation trial (*$P < 0.05$, two-way RM ANOVA with Tukey *post-hoc* test), but not in subsequent post-stimulation responses ($P > 0.05$, two-way RM ANOVA). Middle: no significant differences in average onset latency of evoked spikes were observed within or between vehicle and 7-NI-treated groups ($P > 0.05$; two-way RM ANOVA with one factor repetition). Bottom: train stimulation decreased the standard deviation of spike latency of cortically evoked spikes to a similar degree in both vehicle- and 7-NI-treated animals ($^{\#}P < 0.05$; two-way RM ANOVA with one factor repetition). Note that all recording trials were performed in series (pre-train, train, post-1,2,3) and consisted of single-pulse stimulation delivered for 200 s with the exception of the train stimulation trial (50 s). Error bars indicate SEM of group measures taken from all neurons recorded in between-subjects studies (29 cells recorded in 13 vehicle-treated rats and 29 cells recorded in 11 7-NI-treated rats).

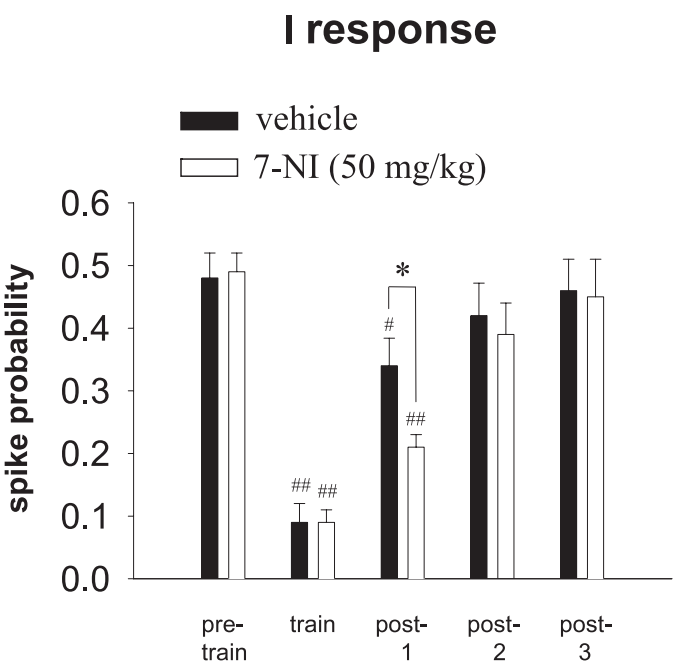

FIG. 8. Impact of nNOS inhibition on striatal neurons exhibiting an I response during train stimulation of the frontal cortex. Within- and between-group comparisons of cortically evoked spike activity in the subpopulation of neurons responding to train stimulation with an I response revealed significant differences resulting from both train stimulation and 7-NI treatment ($P < 0.05$; two-way RM ANOVA with one factor repetition). In both vehicle- and 7-NI-treated control animals, train stimulation had consistent inhibitory effects on cortically evoked spike activity assessed during the train stimulation trial ($^{\#\#}P < 0.001$; two-way RM ANOVA with one factor repetition), and also significantly depressed evoked spike activity in the first post-train stimulation trial ($^{\#\#}P < 0.001$, $^{\#}P < 0.05$, two-way RM ANOVA with Tukey *post-hoc* test). Vehicle and 7-NI administration did not alter subsequent poststimulation responses (post-2, post-3) to single pulse stimulation of the cortex ($P > 0.05$; two-way RM ANOVA with one factor repetition). Between-group comparisons revealed that pretreatment with 7-NI potentiated the short-term depression of cortically evoked spike activity observed in the first post-train stimulation trial ($^{*}P < 0.05$, two-way RM ANOVA with Tukey *post-hoc* test). Error bars indicate SEM of group measures derived from 12 cells recorded following vehicle administration and 21 cells recorded following 7-NI administration.

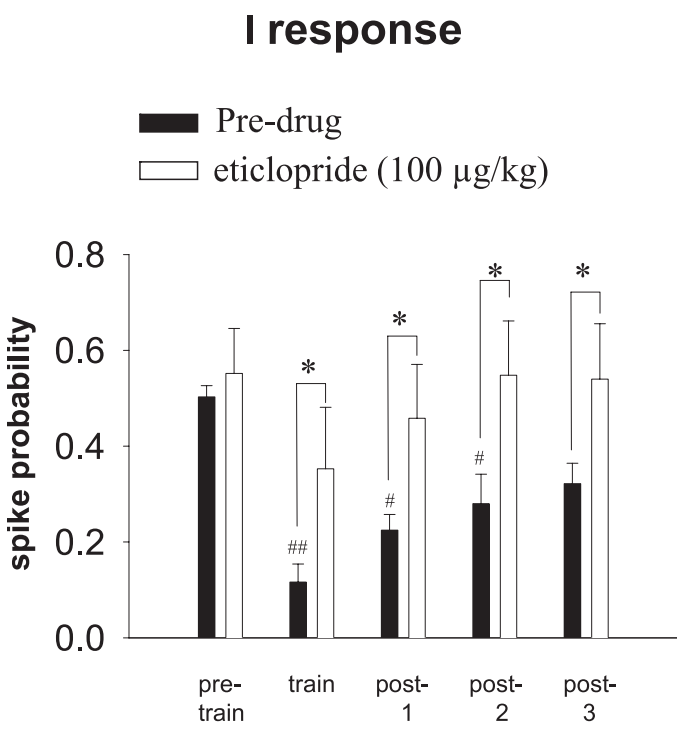

FIG. 9. Impact of D2 receptor antagonism on striatal neurons exhibiting an I response during train stimulation of the frontal cortex. Within- and between-group comparisons of cortically evoked spike activity measured prior to train stimulation (pre-train), during train stimulation (train) and following train stimulation (post 1–3) revealed significant differences resulting from both train stimulation and eticlopride treatment ($P < 0.05$; two-way RM ANOVA with two factor repetition). Prior to eticlopride administration, train stimulation of the contralateral cortex in a manner identical to that used to evoke NO efflux, significantly depressed evoked spike activity during the train and in the first and second post-train stimulation trials ($^{\#\#}P < 0.001$, $^{\#}P < 0.05$, two-way RM ANOVA with Tukey *post-hoc* test). Administration of eticlopride (100 μg/kg, i.p.) reduced the attenuation of cortically evoked spike activity observed during the train stimulation trial ($^{*}P < 0.05$, two-way RM ANOVA) and in the first and second post-train stimulation trials ($^{*}P < 0.05$, two-way RM ANOVA with Tukey *post-hoc* test). Error bars indicate SEM of group measures derived from 11 neurons/rats recorded prior to and following eticlopride administration.

response depended on time in relation to the train stimulation trial. Cortically evoked spike activity was strongly depressed to a similar degree in both groups during the train stimulation trial (Fig. 8, $P < 0.001$). Interestingly, 7-NI administration potently increased the short-term depression of cortically evoked activity observed in the first post-train stimulation trial beyond that exhibited in vehicle-treated controls (Fig. 8, $P < 0.05$), but did not alter subsequent post-stimulation responses (Fig. 8, $P > 0.05$).

Impact of electrical train stimulation of the contralateral frontal cortex on cortically evoked activity: effects of dopamine D2 receptor antagonist administration

Numerous studies have reported that dopamine D2 receptor agonism plays a critical role in the regulation of corticostriatal synaptic plasticity and long-term depression (for a review, see Calabresi *et al*., 2007). Thus, the role of D2 receptor activation in mediating the short-term depression of cortically evoked activity observed in post-train trials in the current study was assessed following systemic administration of the selective D2 receptor antagonist eticlopride (Sammut *et al*., 2007a). To determine the impact of D2 receptor activation on the magnitude of inhibition observed during train stimulation and the short-term depression of post-train spike activity, within-group comparisons of cortically evoked spike activity, recorded prior to and following eticlopride administration (100 μg/kg, i.p., 20 min), were performed on neurons exhibiting an I response. Pre-drug comparisons of measures assessed prior to train stimulation (pre-train), during train stimulation (train) and following train stimulation (post 1–3) revealed multiple significant overall effects of train stimulation of the contralateral cortex across time (Fig. 9, $F_{4,40} = 6.597$, $P < 0.001$; two-way RM ANOVA with two factor repetition, $P < 0.05$ in all cases, Tukey *post-hoc* test). Moreover, eticlopride administration was observed significantly to modify the effects of stimulation (Fig. 9, $F_{1,40} = 5.877$, $P < 0.05$; two-way RM ANOVA with two factor repetition, $P < 0.05$ in all cases, Tukey *post-hoc* test). Similar to the above studies with 7-NI, prior to eticlopride administration train stimulation of the contralateral cortex induced a short-term depression of cortically evoked activity in the first and second post-train stimulation trials (Fig. 9, $P < 0.05$) but did not significantly alter the final (post 3) post-train stimulation response (Fig. 9, $P > 0.05$). Interestingly, eticlopride administration attenuated the inhibition of cortically evoked activity observed during train stimulation of the contralateral cortex (Fig. 9, $P < 0.05$) and reduced the short-term depression of cortically evoked

activity observed in the first and second post-train stimulation trials (Fig. 9, $P < 0.05$). No significant difference in average onset latency of evoked spikes was observed following eticlopride administration (data not shown).

Discussion

A major finding of our study is that activation of nitrergic transmission during train stimulation of the contralateral cortex exerts a powerful and transient feed-forward excitatory influence on spike activity evoked via single-pulse stimulation of the ispilateral cortex. This facilitation of nitrergic signaling opposes concurrent inhibitory influences mediated by dopamine D2 receptor activation. These findings are consistent with our recent work examining the impact of NOS inhibition on up and down state activity (West & Grace, 2004) and local field potentials (Sammut *et al*., 2007b) recorded in the intact striatum. A hypothetical diagram depicting this potential feed-forward regulation is shown in Fig. 10. This model is supported by observations that partial inhibition of nNOS activity following 7-NI administration induced a large attenuation of cortically evoked activity during and immediately after train stimulation of the contralateral cortex. Additionally, excitatory responses observed during train stimulation were abolished in 7-NI-treated animals. 7-NI administration also significantly decreased spontaneous firing activity without affecting measures of spike activity evoked by single-pulse stimulation of the ipsilateral frontal cortex. These results indicate that while tonic NO signaling facilitates the spontaneous firing activity of striatal MSNs, phasic NO transmission, stimulated by synchronous burst firing of corticostriatal inputs, plays a major role in facilitating cortically evoked spike activity in a manner which offsets concurrent dopamine D2 receptor-mediated inhibitory influences on striatal MSNs (Fig. 10).

Technical considerations

In the current study, the termination sites of all recording electrode tracks in the dorsal striatum were verified using standard histological methods (see Results; Sammut *et al*., 2007b). In single-unit recording studies all neurons were isolated during electrical stimulation of the

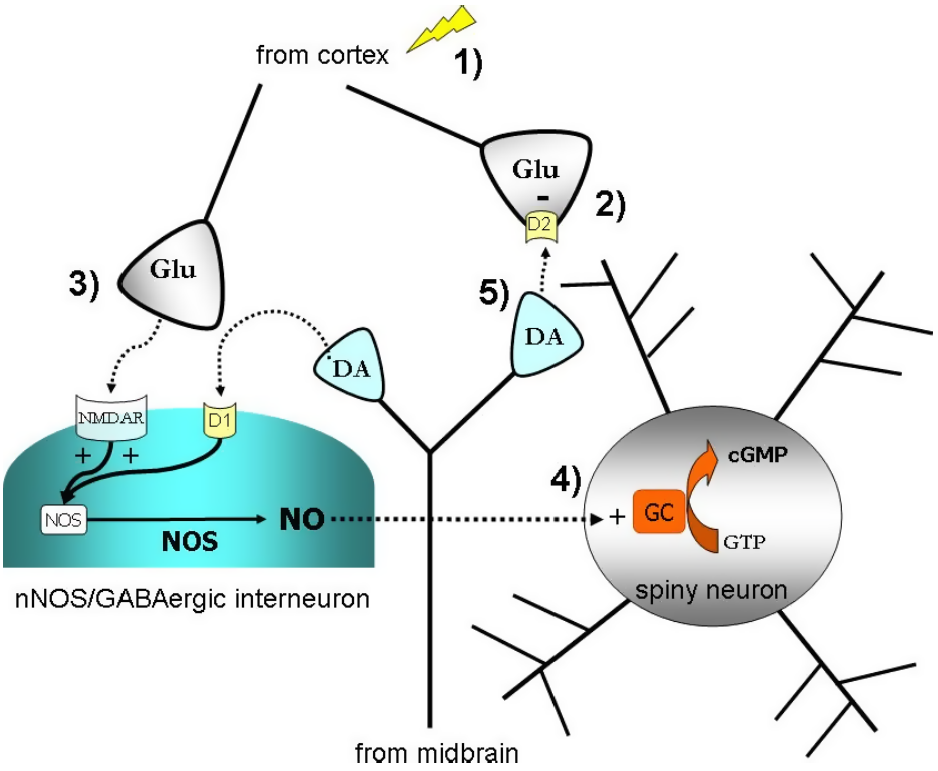

FIG. 10. Schematic of proposed feed-forward regulation of striatal MSN activity by frontal cortical afferents and striatal nNOS interneurons. Previous studies have shown that excitatory corticostriatal inputs synapse directly onto the heads of dendritic spines of striatal MSNs (for review, see Wilson, 2004). Frontal corticostriatal inputs also target the dendrites of striatal nitrergic/GABAergic interneurons (Kawaguchi, 1997). In addition to the nNOS interneurons, the parvalbumin expressing fast-spiking interneurons (not shown) also use GABA as a transmitter (Kawaguchi, 1993). In this model, derived from the current studies and previous work described herein, we propose that coherent phasic activation of frontal cortical afferents (1) exerts direct excitatory effects on MSNs (2). Concurrently, robust activation of corticostriatal inputs to nNOS-positive interneurons (3) increases nitrergic transmission via NMDA receptor activation (Sammut *et al*., 2007b). The activation of nNOS interneurons may require concurrent D1 receptor activation (Calabresi *et al*., 2007). Phasic activation of NO signaling increases MSN excitability (4), possibly via a GC-dependent mechanism (West & Grace, 2004). The excitatory influence of phasic NO signaling is attenuated by dopamine mediated-D2 receptor activation which acts to dampen subsequent synaptic responses in MSNs (5). Activation of corticostriatal inputs is also likely to stimulate feed-forward inhibition mediated by both fast-spiking interneurons and nitrergic/GABAergic interneurons (not shown; Tepper *et al*., 2004). In support of this model, the current results showed that systemic administration of an nNOS inhibitor attenuated striatal NO efflux and induced a large decrease in cortically evoked spike activity of striatal neurons during, and immediately after, train stimulation of the contralateral cortex. Given these observations, it is likely that disruption of excitatory nitrergic signaling would result in an increase in the impact of competing inhibitory influences on MSNs. Consistent with this prediction, systemic administration of the nNOS inhibitor increased the incidence of I responses and eliminated E responses during train stimulation of the frontal cortex. Following nNOS inhibitor administration, the magnitude of D2 receptor-mediated short-term depression of cortically evoked spike activity was also increased in a subpopulation of cells responding to train stimulation with an I response. Under physiological conditions, it is likely that cortical afferents would control feed-forward inhibitory and excitatory processes differentially during the coordination of goal-directed motor behavior. Similar feed-forward mechanisms have been shown to play important roles in the regulation of short-term plasticity in other neural microcircuits such as the hippocampus (Silberberg *et al*., 2005).

frontal cortex and exhibited cortically evoked, sometimes spontaneous activity that was consistent with the electrophysiological properties of striatal MSNs identified in previous studies using morphological techniques and antidromic activation (Mallet *et al*., 2005, 2006).

Given the complexity of cortex–basal ganglia loops, it is possible that the observed increase in striatal NO efflux evoked via contralateral train stimulation resulted from activation of both corticostriatal and other indirect pathways. In addition, the NOS inhibitors and D2 antagonist used in the current study were all delivered systemically to intact urethane anesthetized rats. Thus, it is possible that some of the effects of the drug occurred via the disruption of extrastriatal NO signaling or other mechanisms. However, the lack of effect of 7-NI on activity evoked by single-pulse cortical stimulation indicates that partial nNOS inhibition did not have a major impact on the activation of cortical afferents. This observation, together with the finding that decreased striatal NO efflux following 7-NI administration eliminated short-latency E responses evoked during train stimulation, indicates that the excitatory effects of phasic NO transmission on striatal MSNs are likely to be mediated by a direct activation of nNOS-containing interneurons by corticostriatal inputs (see Fig. 10).

Regulation of striatal neuron activity by tonic NO signaling

Very low ambient concentrations of 'tonic' NO can be measured in many brain regions, including the hippocampus (Hopper & Garthwaite, 2006), cerebellum (Roy & Garthwaite, 2006) and striatum (Ohta *et al*., 1994; Globus *et al*., 1995). Recent studies provide support for a role of tonic NO in activating GC and depolarizing neurons via the generation of cGMP (West & Grace, 2004; Podda *et al*., 2004; Garthwaite *et al*., 2006). Our previous studies of tonic NO signaling in the striatum found that ambient levels of NO acted to increase significantly the membrane excitability of striatal MSNs. Thus, disruption of endogenous NO–GC signaling via intrastriatal or intracellular application of antagonists/inhibitors was found to decrease the amplitude of depolarized 'up events' and EPSPs as well as the membrane excitability of striatal neurons exhibiting electrophysiological and morphological characteristics of MSNs (West & Grace, 2004). Large decreases in input resistance were also observed in studies using either an NO scavenger or a GC inhibitor (West & Grace, 2004). Importantly, in both cases the decrease in input resistance was at least partially attenuated by co-administration of a cGMP analog, suggesting that tonic NO–GC signaling may play an important role in regulating ion channels involved in controlling the membrane excitability of MSNs (West & Grace, 2004). In support of this, the current study showed that systemic administration of the nNOS inhibitor 7-NI strongly inhibited the spontaneous firing activity of striatal neurons isolated during single-pulse cortical stimulation. These findings indicate that like GABAergic, cholinergic and dopaminergic modulation (Nisenbaum & Berger, 1992; Tseng *et al*., 2001; Mallet *et al*., 2005, 2006), tonic NO signaling may play an important role in regulating spike activity in striatal MSNs recorded *in vivo*.

In contrast to our results, a previous study reported that systemic 7-NI, administered using the same dose and route used in the current study, increased the basal firing rate of 12 striatal neurons recorded in urethane-anesthesthized rats (Sardo *et al*., 2002). Although it is unclear why disparate findings were observed between the studies, differences in signal filtering, isolation of single units, and sampling of striatal subpopulations may have contributed significantly to observed outcomes. In the current study, only single units responding to frontal cortical stimulation with short-latency spikes consisting of bi- (+/–) or tri-phasic (+/–/+) waveforms were included in the data set (see Figs 2 and 3). The isolation of single units using cortical stimulation also allowed us to eliminate potential fast-spiking interneurons from the data set (Mallet *et al*., 2005, 2006). In contrast, Sardo *et al*. (2002) recorded spontaneously active neurons exhibiting negative/positive waveforms and did not examine cortically evoked activity. As indicated above, the current findings are consistent with previous studies examining the impact of NOS inhibitors on the subthreshold membrane activity of identified MSNs.

Facilitation of phasic NO efflux by frontal cortical afferents

We have previously shown that NO efflux is robustly increased *in vivo* in a frequency- and stimulus intensity-dependent manner following train stimulation of the frontal cortex (Sammut *et al*., 2007b). In these studies, low-frequency (3 Hz) electrical stimulation of frontal cortical afferents did not consistently evoke detectable changes in striatal NO levels, whereas train stimulation (30 Hz, 800-ms train duration, 2.0-s ITI for a duration of 100 s) facilitated NO efflux via NMDA receptor–nNOS-dependent mechanisms (Sammut *et al*., 2007b). The current studies extend these findings by showing that contralateral train stimulation (30 Hz, 1000-ms train duration, 2.0-s ITI for a duration of 50 s) of the frontal cortex increases striatal NO efflux in a transient manner. Additionally, evoked NO efflux was not altered by vehicle administration and was stable across multiple stimulation trials (∼every 20 min) for at least 2 h. Importantly, this facilitation of NO efflux was sensitive to nNOS inhibitor administration.

Consistent with previous findings (Eblen *et al*., 1996; Kalisch *et al*., 1996; Adachi *et al*., 2000; Sammut *et al*., 2007b), the maximal inhibition of NOS activity observed following systemic administration of large doses of NOS inhibitors in the current studies was < 60%. It is unclear why both nNOS selective and non-selective NOS inhibitors exhibit poor efficacy when administered systemically. Given that the studies cited above observed relatively consistent results with regard to magnitude of NOS inhibition, the modest efficacy of these inhibitors may result from poor penetration into the brain and/or competition with endogenous L-arginine for the nNOS active site (Ohta *et al*., 1994).

Regulation of cortically evoked activity by phasic NO signaling and D2 receptor activation

As outlined above, electrophysiological recordings showed that pharmacological disruption of phasic NO efflux evoked during train stimulation increased the magnitude of short-term depression of cortically evoked spike activity, possibly due to decreased feed-forward excitation by NO interneurons (Fig. 10). This, in turn, may have resulted in the observed increase in the incidence of I responses and greater dopamine-mediated inhibition of cortically evoked spike activity in the first post-train stimulation trial. This is supported further by the complete lack of E responses observed in animals treated with 7-NI (Table 1). Studies using the dopamine D2 receptor antagonist eticlopride indicated that the inhibition of cortically evoked spike activity observed during the train and in post-train stimulation trials were mediated via D2 receptor activation. Numerous studies have shown that electrical stimulation of frontal cortical afferents increases subcortical dopamine release (for a review, see West *et al*., 2003). Additionally, D2 receptors have been shown to exert a tonic inhibitory influence over corticostriatal glutamatergic afferents (Hsu *et al*., 1995; Umemiya & Raymond, 1997; West & Grace, 2002; Bamford *et al*., 2004) and play a critical role in the long-term depression of striatal synaptic transmission (for a review, see Calabresi *et al*., 2007). To our knowledge, this is the first report demonstrating that D2 receptor activation mediates the short-term depression of cortically evoked

spike activity of striatal neurons *in vivo*. We have shown previously that activation of NO synthesis is also down-regulated via a dopamine D2 receptor-dependent mechanism (Sammut *et al*., 2007a). Thus, D2 receptor activation may act in opposition to striatal NO signaling on several mechanistic levels.

Multiple studies have used either reverse microdialysis or microiontophoresis to examine the impact of locally applied NO generators or NOS inhibitors on evoked firing activity of striatal neurons (West *et al*., 2002; Di Giovanni *et al*., 2003; Liu *et al*., 2005b). Phasic activation of NO signaling via intrastriatal infusion of the NO-generating compound SNAP increased the firing rate and burst activity of striatal neurons under basal conditions and during single-pulse electrical stimulation of the prefrontal cortex (West *et al*., 2002). Similar to these findings, microiontophoresis of the NO generator sodium nitroprusside was shown to increase the basal firing rate of 51 out of 66 striatal neurons and to potentiate the excitatory effects of glutamate on a subpopulation of neurons (Liu *et al*., 2005a). This same study reported that microiontophoresis of the NOS inhibitor L-NAME decreased the excitatory effects of glutamate on a subpopulation of neurons (Liu *et al*., 2005a). In contrast to the above studies, microiontophoresis of the superoxide and NO generator 3-morpholinosydnonimine (SIN-1) was shown to decrease glutamate-evoked firing in 12 of 15 neurons, whereas L-NAME increased the excitatory effects of glutamate on striatal neurons (Di Giovanni *et al*., 2003). Given that SIN-1 generates superoxide concurrently with NO, which together react to form peroxynitrite spontaneously (Hogg *et al*., 1992), it is possible that the inhibitory effects observed in the later study were not related to NO generation, but resulted via the actions of another reactive oxygen species. It is unclear why these studies found opposite effects of L-NAME on glutamate-evoked firing. However, the study by Liu and colleagues tested the effects of L-NAME on a significantly larger sample of striatal neurons (28 cells) as compared with that of Di Giovanni and colleagues (ten cells).

The current findings are also consistent with our recent study of the impact of train (30 Hz) stimulation of the frontal cortex on striatal function using dual NO microsensor and local field potential recordings (Sammut *et al*., 2007b). Although stimulation artifacts prohibited measurements of local fields during the train stimulation trial, we were able to assess the impact of NOS inhibition on activity recorded in a post-train trial (0–30 s) that is comparable with the post 1 stimulation trial (0–200 s) described herein (Sammut *et al*., 2007b). The results of these studies demonstrated that systemic administration of the NO synthase/GC inhibitor methylene blue simultaneously attenuated evoked NO efflux and the peak oscillation frequency (within the delta band) of local striatal field potentials recorded immediately after cortical train stimulation (Sammut *et al*., 2007b). These findings are consistent with previous studies showing that MSNs recorded during intrastriatal infusion of a NO scavenger exhibited smaller facilitatory synaptic responses to paired electrical stimuli delivered to the prefrontal cortex as compared with control neurons (West & Grace, 2004). Taken together, these observations indicate that glutamatergic transmission activated during corticostriatal cell burst firing may be amplified by NOS interneurons involved in synchronizing striatal network activity and spike generation of MSNs recorded in intact striatal networks.

In contrast to our findings, studies by Calabresi and colleagues using brain slice preparations mainly predict an inhibitory action (long-term depression) of the NO–GC signaling pathway on the synaptic activity of striatal MSNs (Calabresi *et al*., 1999a,b, 2000). It is possible that the apparent differences between these studies performed *in vitro* and our studies performed in the intact animal are related to differences in the individual network properties of neurons within these preparations (Paré *et al*., 1998). Interestingly, stimulation protocols that produce long-term depression *in vitro* have been shown to produce long-term potentiation of corticostriatal neurotransmission *in vivo* (Charpier & Deniau, 1997), possibly due to the greater removal of the voltage-dependent magnesium block of NMDA receptors and higher levels of basal excitability inherent to the intact animal. Lastly, long-term potentiation induced by tetanic stimulation of corticostriatal pathways is reported to be significantly reduced in $eNOS^{-/-}$ mice or following treatment with a non-selective NOS inhibitor (Doreulee *et al*., 2003). Thus, in addition to the preparation, the role played by NO in striatal synaptic plasticity may depend on the stimulation protocol and the recruitment of different NOS isoforms.

Functional implications

The facilitation of corticostriatal throughput by phasic NO signaling may represent a mechanism by which the impact of robust corticostriatal signaling on MSNs is strengthened by feed-forward striatal integration (Fig. 10). Similar interneuron-dependent feed-forward excitatory processes have been demonstrated in the hippocampus and spinal cord (for a review, see Silberberg *et al*., 2005) as well as the basolateral amygdala (Woodruff *et al*., 2006). Support for this novel network property of striatal NOS interneurons comes from studies showing that train stimulation of corticostriatal pathways facilitates electrotonic coupling between MSNs in striatal slice preparations via an NO-dependent process (O'Donnell & Grace, 1997). An NO-mediated synchronization of electrotonic coupling, together with facilitation of glutamatergic transmission, may be important for promoting and/or synchronizing up-state activity and spike discharge of a network of functionally related MSNs. Consistent with these observations, studies described above (West & Grace, 2004) revealed that inhibition of NOS and GC depressed the amplitude of spontaneous up events known to be driven by glutamateric inputs (O'Donnell & Grace, 1995; Wilson & Kawaguchi, 1996). Our current findings, together with the above observations, suggest that a dysfunction in striatal nitrergic neurotransmission could disrupt the operation of the basal ganglia by altering corticostriatal information processing and short-term plasticity in MSNs and result in inactivation of striatal output circuits. In support of this, a recent study utilizing quantitative EEG methods has shown that inhibition of neuronal NOS activity has a major impact on electrical activity patterns within corticostriatopallidal circuits, indicating that NO signaling plays an important role in modulating efferent pathways to the globus pallidus (Ferraro *et al*., 2002). Future studies examining the impact of tonic and phasic NO transmission on identified striatonigral and striatopallidal neurons should help to clarify the neuromodulatory impact of this important neural messenger on striatal function.

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Abbreviations

7-NI, 7-nitroindazole; E, excitatory; FHC, Frederick Haer Company; GC, guanylyl cyclase; I, inhibitory; ITI, intertrain interval; MSNs, medium spiny neurons; L-NAME, N^G-nitro-L-arginine methyl ester hydrochloride; NMDA, *N*-methyl-D-aspartame; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, NO synthase; PBS, phosphate-buffered saline; PSTH, peri-stimulus time histograms; SNAP, (+/−)*S*-nitroso-*N*-acetyl-penicillamine; WPI, World Precision Instruments.

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