GABAergic Afferents Activate Both GABA$_A$ and GABA$_B$ Receptors in Mouse Substantia Nigra Dopaminergic Neurons *In Vivo*

Elena Brazhnik, Fulva Shah, and James M. Tepper

Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, New Jersey 07102

Most *in vivo* electrophysiological studies of substantia nigra have used rats. With the recent proliferation of the use of mice for *in vitro* neurophysiological studies because of the availability of various genetically modified strains to identify the roles of various channels and proteins in neuronal function, it is crucial to obtain data on *in vivo* responses in mice to verify that the *in vitro* results reflect functioning of systems comparable with those that have been well studied in rat.

Inhibitory responses of rat nigral dopaminergic neurons by stimulation of afferents from striatum, globus pallidus, or pars reticulata have been shown to be mediated predominantly or exclusively by GABA$_A$ receptors. This is puzzling given the substantial expression of GABA$_B$ receptors and the ubiquitous appearance of GABA$_B$ synaptic responses in rat dopaminergic neurons *in vitro*. In the present study, we studied electrically evoked GABAergic inhibition in nigral dopaminergic neurons in C57BL/6J mice. Stimulation of the three major GABAergic inputs elicited stronger and longer-lasting inhibitory responses than those seen in rats. The early inhibition was GABA$_A$ mediated, whereas the later component, absent in rats, was GABA$_B$ mediated and selectively enhanced by GABA uptake inhibition. Striatal-evoked inhibition exhibited a slower onset and a weaker initial component compared with inhibition from globus pallidus or substantia nigra pars reticulata. These results are discussed with respect to differences in the size and neuronal density of the rat and mouse brain and the different sites of synaptic contact of the synapses from the three GABAergic afferents.

Key words: substantia nigra; dopaminergic; inhibition; striatonigral; pallidonigral; pars reticulata

Introduction

Midbrain dopaminergic neurons have long been recognized to play an essential role in the execution of voluntary movement and in the pathophysiology of Parkinson's disease (Hornykiewicz, 1998), to be involved in schizophrenia and in mechanisms of action and addiction to of drugs of abuse (Diana and Tepper, 2002; Kauer and Malenka, 2007), as well as to underlie critical aspects of higher cognitive functions including some types of motor learning, sensorimotor integration, and signaling reward-related aspects of sensory stimuli (Aosaki et al., 1994; Schultz, 1998, 2007).

Most of the afferents to the substantia nigra are GABAergic, and at least 70% of the synapses formed on nigral dopaminergic neurons are GABAergic (Ribak et al., 1976; Bolam and Smith, 1990). Most of the GABAergic innervation arises from the striatum (Grofová and Rinvik, 1970; Hattori et al., 1973a,b; Somogyi et al., 1981; Bolam and Smith, 1990), the globus pallidus (GP) (Hattori et al., 1975; Smith and Bolam, 1990), and the local axon collaterals of the substantia nigra pars reticulata projection neurons (SNr) (Deniau et al., 1982; Grofova et al., 1982; Nitsch and

Riesenberg, 1988; Hajós and Greenfield, 1993, 1994; Tepper et al., 1995; Mailly et al., 2003) (for recent review, see Tepper and Lee, 2007).

Neurons in substantia nigra pars compacta and pars reticulata express GABA$_A$ and GABA$_B$ receptors (Bowery et al., 1987; Nicholson et al., 1992; Charara et al., 2000; Boyes and Bolam, 2003), and nigral neurons respond to stimulation of GABA$_A$ and/or GABA$_B$ receptors with hyperpolarization and/or inhibition of firing *in vivo* (Grace and Bunney, 1979; Erhardt et al., 2002) or *in vitro* (Pinnock, 1984; Lacey et al., 1988; Gulácsi et al., 2003). However, although local electrical stimulation with high-frequency trains *in vitro* evokes both GABA$_A$ and GABA$_B$ IPSPs/IPSCs (Sugita et al., 1992; Johnson and North, 1992; Cameron and Williams, 1993; Hajós and Greenfield, 1993, 1994; Häusser and Yung, 1994; Saitoh et al., 2004), in early studies, striatal stimulation *in vivo* produced inhibition and IPSPs that appeared to be mediated exclusively by GABA$_A$ receptors (Nakamura et al., 1979; Grace and Bunney, 1985). Subsequent studies showed that inhibition resulting from stimulation of striatal, pallidal, or pars reticulata afferents was substantially or completely blocked by local application of GABA$_A$ antagonists but was not attenuated at all by selective GABA$_B$ antagonists, suggesting mediation predominantly or exclusively by GABA$_A$ receptors (Tepper et al., 1995; Paladini et al., 1999). The reasons for these differences remained unexplained.

During recent *in vivo* recordings from dopaminergic neurons

in wild-type controls for transgenic R6/2 mice (Shah et al., 2005), we observed that striatal stimulation produced significantly longer-lasting inhibition than comparable stimulation in rats (Paladini et al., 1999), suggesting the possibility of a GABA_B response. Therefore, we decided to reinvestigate the roles of GABA_A and GABA_B receptors and the effects of GABA uptake blockade in afferent-induced inhibition of dopaminergic neurons *in vivo* in mice.

Materials and Methods

General surgery. All procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Rutgers Animal Care and Facilities Committee. Thirty-five male C57BL6/J mice, obtained from The Jackson Laboratory and weighing between 20 and 30 g at the time of recording, were used as subjects. Mice were housed two per cage in plastic shoebox cages at 21°C on a 12 h light/dark cycle with food and water available *ad libitum*.

Mice were anesthetized with chloral hydrate (350 mg/kg, i.p.) and installed into a stereotaxic frame. Heart rate and respiration rate were monitored, and supplemental injections of chloral hydrate were given ~1 h through an indwelling intraperitoneal catheter to maintain an even level of anesthesia. All wound margins and point of contact between the animal and the stereotaxic apparatus were infiltrated with lidocaine solution (2%). Body temperature was maintained at 37°C.

The surgery, extracellular recording, and local injection of drugs have been described previously (Paladini et al., 1999). Briefly, after subcutaneous injection of 5% bupivacaine with epinephrine, the scalp was removed and small burr holes were drilled over the appropriate coordinates, for striatum [anterior (A), 0.9 mm from bregma; lateral (L), 2.3 mm from midline], GP (A, −0.4 mm from bregma; L, 1.8 mm), and ventral thalamus (VT) (A, −1.0 mm from bregma; L, 1.2) for antidromic activation of SNr projection neurons (Tepper et al., 1995). Two stimulating electrodes were implanted in two different regions in each mouse. A 2 mm hole for insertion of the recording microelectrode was drilled above substantia nigra at the following coordinates: A, 1.3 mm from lambda; L, 1.2 from midline.

Stimulation, recording, and drug infusion. Bipolar stimulating electrodes consisting of two stainless steel enamel-coated wires (California Fine Wire) ~100 μm in diameter with a tip separation of ~100 μm were lowered to the appropriate depths in striatum (−2.5 mm from surface), GP (−3.5 mm), and thalamus (−3.5 mm) and affixed in place with cyanoacrylate glue and dental cement. Constant current electrical stimuli were generated with Winston A-65 timer and SC-100 constant-current stimulus isolation units (Winston Electronics). Stimuli consisted of single monophasic square wave pulses ranging in intensity from 100 to 700 μA with durations of 100 μs at a rate of 1.0 Hz. Train stimuli consisted of five 100–500 μA, 100-μs-duration pulses delivered over 50 ms (100 Hz). Trains were delivered at 1.0 Hz. At the end of the experiment, stimulating sites were marked with a small lesion made by passing a 1.0 mA direct current through the stimulating electrode.

Recording electrodes were made from 2.0 mm outer diameter capillary tubing (WPI) using a Narishige PE-2 vertical pipette puller. The electrodes, whose tips were broken under microscopic control to yield final tip diameter of 1.0–2.0 μm, typically possessed resistances between 5 and 15 MΩ *in vivo*. Barrels for micropressure ejection of drugs were pulled from 1 mm outer diameter borosilicate glass tubing (Fisher Scientific), and their tips were broken back under microscopic control to final diameters of 8–10 μm. The barrels were then heated and bent to ~30° of vertical shaft. Two pressure ejection pipettes were aligned with the recording electrode shaft with their tips 50–70 μm behind the tip of recording electrode under microscopic control and affixed with cyanoacrylate glue. Before the experiment, the recording electrode was filled with 1 M NaCl, and pressure ejection barrels were filled with GABA antagonists: picrotoxin (PTX) (400–1000 μM in 0.9% saline solution; formerly Research Biochemicals, now Sigma) or CGP55845A (CGP) [(2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenyl-

methyl)phosphinic acid] (500–1000 μM in 0.9% saline solution; Tocris Cookson).

Single-unit extracellular recordings were amplified with a Neurodata IR183 preamplifier and displayed on a Tektronix 5113A storage oscilloscope. Data were digitized at 40 kHz with a CED Micro1401 Mk II (Cambridge Electronic Design) and acquired on a personal computer running Spike2 software (Cambridge Electronic Design) and stored for later offline analysis. Drugs were applied locally through the side barrels by pressure ejection for a period of 100–500 ms at 20–40 psi with a Picospritzer (General Valve Corporation). Drugs were applied in a counterbalanced manner after predrug data were collected. The onset of drug effects was typically within 1–3 s, and drug effects lasted for ~15–30 min. Although no attempt was made to reverse the effects of the GABA receptor antagonists pharmacologically in these experiments, previous studies using the same method of pressure application showed that the local pressure application of GABA agonists was reversible during subsequent pressure application of appropriate antagonists (Paladini and Tepper, 1999; Paladini et al., 1999).

In all experiments, data were obtained from only one neuron per track to avoid residual drug effects. In some cases, GABA receptor antagonists were applied to up to three neurons from one animal, with recording tracks separated by at least 500 μm. For evoked responses, stimulation intensities were adjusted for each stimulating region. Striatal and pallidal afferents to substantia nigra dopaminergic neurons were activated by electrically stimulating within those nuclei, whereas SNr afferents to dopaminergic neurons were monosynaptically activated by electrically stimulating the ipsilateral thalamus thereby inducing antidromic activation selectively of a large population of pars reticulata GABAergic projection neurons. This excitation propagates into the local axon collaterals that synapse onto dopaminergic neurons (Tepper et al., 1995, 2003). For the sake of brevity, this will be referred to as SNr stimulation, although it should be understood that the stimulating electrode is in the ventral thalamus, and the SNr projection neurons are antidromically activated.

Initial currents were ~100 μA and were gradually increased until a clear and reliable inhibitory response was obtained, being careful to keep the stimulating current below the minimal amplitude that evoked antidromic responses for each neuron. After reaching this point, at least 5 min of evoked inhibition were recorded to establish the stability of the response. Then the drugs were administered, and, after 1 min of recording of spontaneous activity to define alterations in firing rate and firing patterns, stimulation was applied again using the same current intensities that were used during baseline sampling. For single-pulse stimulation, 100–200 sweeps were typically recorded before and again after drug application, whereas for train stimulation typically 40–100 sweeps were obtained before and after drug application. We tested the effect of two GABAergic antagonists: picrotoxin, a GABA_A receptor antagonist, and CGP55845A, a selective GABA_B receptor antagonist. Typically two to five pressure applications were applied per drug. All drugs were dissolved in physiological saline at high concentration, stored in aliquots at −20°C, and used within 1 month. The final concentrations of these solutions were adjusted before the experiment. The effective concentrations of these drugs were determined empirically in previous studies (Celada et al., 1999; Paladini and Tepper, 1999; Paladini et al., 1999).

In some experiments, the effects of intraperitoneal injection of the specific GAT-1 blocker NO-711 [1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,4,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride] (1 mg/kg; Tocris Cookson) (Borden et al., 1994) on spontaneous activity and afferent-induced inhibition were explored, whereas in others (n = 7), local pressure application of nipecotic acid (1 mM; Aldrich) (Krogsgaard-Larsen, 1980). In both cases, the hope was that this would increase electrically evoked synaptic levels of GABA enough to overflow the synapse and activate extrasynaptic GABA_B receptors (Scanziani, 2000).

Histology. At the end of each experiment, the mouse was injected with lethal dose of chloral hydrate, perfused transcardially with saline followed by 10% Formalin. The brains were removed, postfixed in Formalin, cut into 50 μm coronal sections on a freezing microtome, and stained with neutral red to enable histological verification of recording and stimulating electrode sites.

Data analysis. Data were analyzed off-line using Spike2. Peristimulus time histograms (PSTH) and autocorrelograms were constructed from samples of spontaneous spike trains of 1000–2000 spikes. Firing patterns were classified as pacemaker, random, or bursty from the autocorrelograms as described previously (Tepper et al., 1995). PSTHs (400–1000 ms duration with 2 ms bins) were constructed for each cell for the predrug baseline state and after administration of each of the antagonists. Stimulus-induced inhibition and the effects of local drug application were quantified by a Spike2 script written to consider the onset of inhibition as beginning with the first of at least three consecutive bins that fell outside the range of mean ± 1 SD of the average firing rate during the baseline control period. The offset of inhibition was similarly defined as beginning with the first of at least three consecutive bins that was within the range of mean ± 1 SD. Percentage inhibition was calculated as the mean firing rate during the entire period of inhibition as defined above.

Data were analyzed by using one- and two-way ANOVA with Prism (GraphPad Software), followed with Bonferroni's *post hoc* tests to compare individual group means. Differences were considered to be significant if $p < 0.05$. All numerical values are expressed as mean ± SEM.

Results

Data were obtained from 110 spontaneously active substantia nigra dopaminergic neurons that were recorded from 35 mice. All cells reported were located within the substantia nigra pars compacta as confirmed by postmortem histological analysis of electrode tracks. All data reported here were obtained from cells that met well established criteria for electrophysiological identification of substantia nigra dopaminergic neurons in extracellular recordings *in vivo* in anesthetized rats, including low spontaneous firing rates (5.2 ± 0.2 spikes/s; $n = 75$) in a regular, random, or bursty firing pattern (Fig. 1 *D–F*), an extracellularly recorded waveform >2 ms in duration (Fig. 1 *B*) that often exhibited a notch on the initial positive component (Fig. 1 *C*) corresponding to an initial segment–somatodendritic break, and/or long-latency antidromic responses from striatum (10.9 ± 0.6 ms; 23 of 50 neurons, 46%) or GP (6.8 ± 0.5 ms; 22 of 55 neurons, 40%) consisting mostly of initial segment-only spikes. Based on estimated straight-line distances from the striatal and pallidal stimulating sites and the nigral recording location, these correspond to conduction velocities of 0.42 m/s for the nigrostriatal pathway and 0.47 m/s for the nigropallidal pathway, both in excellent agreement with data previously obtained in rat. Example recordings from mouse dopaminergic neurons, illustrated in Figure 1, confirm and extend previous observations in mice (Sanghera et al., 1984; Trulson and Trulson, 1987) and show that these neurons display basic electrophysiological characteristics that are indistinguishable from those previously reported in anesthetized rats *in vivo* (Deniau et al., 1978; Guyenet and Aghajanian, 1978; Grace and Bunney, 1983; Tepper et al., 1984; Paladini et al., 1999).

Responses of substantia nigra dopaminergic neurons to activation of striatal, pallidal, and SNr GABAergic inputs
The periods of poststimulus activity used for the computation of duration and reduction of firing during the inhibitory responses were defined over the same time range regardless of whether we used single pulses or trains. Five neurons that responded antidromically at the stimulation intensities used to elicit orthodromic inhibition (100–600 μA) were excluded from additional analysis because of the possibility of contamination by the long-lasting spike afterhyperpolarization characteristic of these neurons (Grace and Bunney, 1983; Kita et al., 1986; Tepper et al., 1987). To obtain measures of firing rate that would be comparable between the control period and that after administration of

antagonists, we defined the inhibition within the first 100 ms poststimulus as the early inhibitory response (EIR). Inhibition after 100 ms was defined as the late inhibitory response (LIR). Although arbitrary in some respects, the 100 ms cutoff for the EIR and LIR was based on a first pass inspection of our control data as well as a knowledge of the onset latencies and durations reported previously for GABA$_A$- and GABA$_B$-mediated IPSPs in substantia nigra by others (Grace and Bunney, 1985; Hajós and Greenfield, 1993, 1994; Häusser and Yung, 1994)

Inhibitory responses evoked by stimulation of neostriatum, GP, or SNr were occasionally followed by postinhibitory excitation and sometimes by secondary episodes of inhibition at with extremely long-onset latencies (e.g., 375–400 ms) (see Figs. 2*A2*,*B2*, 4*A1*). Onset latencies were analyzed for the EIR but not for the LIR because the onset of the latter often overlapped the EIR as in the examples in Figure 2, *A1*, *A2*, and *B2*.

Responses to striatal stimulation
Responses to single-pulse striatal stimulation were characterized in most neurons by an incomplete inhibition in which the neuron continued to fire but at a substantially lower rate than during the prestimulus baseline period (see Figs. 1*G*, 2*A1*, 4*A*). Occasionally, the stimulation resulted in complete suppression of firing. The onset latency for the EIR was variable, ranging from 6 to 80 ms (mean, 29.1 ± 3.5 ms). The mean duration of the striatal-evoked EIR was 68.1 ± 3.7 ms, during which spontaneous activity was suppressed by 56.9 ± 4.2% compared with the prestimulus level. A majority of dopaminergic neurons (34 of 44, 77%) also exhibited the LIR, examples of which are shown in Figures 2*A* and 4*A*. The mean duration of the LIR was 156.7 ± 11.5 ms and, during the LIR firing, was reduced by 50.9 ± 4.5%.

A high-frequency train of pulses (five pulses at 100 Hz, 100–500 μA, 100 μs) delivered to striatum produced qualitatively similar results as single-pulse stimulation. An EIR was induced in 44 (100%) neurons with an onset latency of 19.7 ± 3.7 ms and a duration of 61.8 ± 3.6 ms, during which firing was reduced by 48.7 ± 3.8%. Similar to single-pulse stimulation, the great majority of neurons (41 of 44, 93%) also exhibited an LIR that was significantly longer in duration (235.7 ± 17.9 ms; $t = 3.652$; $p < 0.001$) and exhibited a greater reduction in firing (by 61.7 ± 3.5%; $t = 2.763$; $p < 0.05$) compared with single-pulse stimulation. Population means of these data are plotted in Figure 3.

Responses to pallidal stimulation
Stimulation of the GP evoked a short-latency EIR in all dopaminergic neurons tested ($n = 58$ cells). Three of these neurons were excluded from additional statistical analysis, one because it exhibited an antidromic response and two others because data on all parameters with both stimulus protocols could not be obtained. Typical examples of an inhibitory response evoked by single-pulse GP stimulation are shown in Figures 1*H* and 2*B*. The EIR exhibited a short onset latency (5.3 ± 0.9 ms), an average duration of 85.2 ± 3.6 ms, during which firing was suppressed by 81.2 ± 3.6% compared with the prestimulus control period. In contrast to striatal-evoked inhibition, pallidal-evoked inhibition always contained an epoch of complete firing suppression (see Figs. 2*B*, 4*B*, 10*B*) that is reflected in a greater mean percentage inhibition (Fig. 3). Also in contrast to striatal stimulation, single-pulse stimulation of GP evoked an LIR in only 26% of neurons (14 of 54 cells) versus 77% (34 of 44) for striatal stimulation. The mean duration of the LIR was 134.2 ± 26.5 ms, during which the firing rate was suppressed by 19.8 + 5.3% of the prestimulus level.
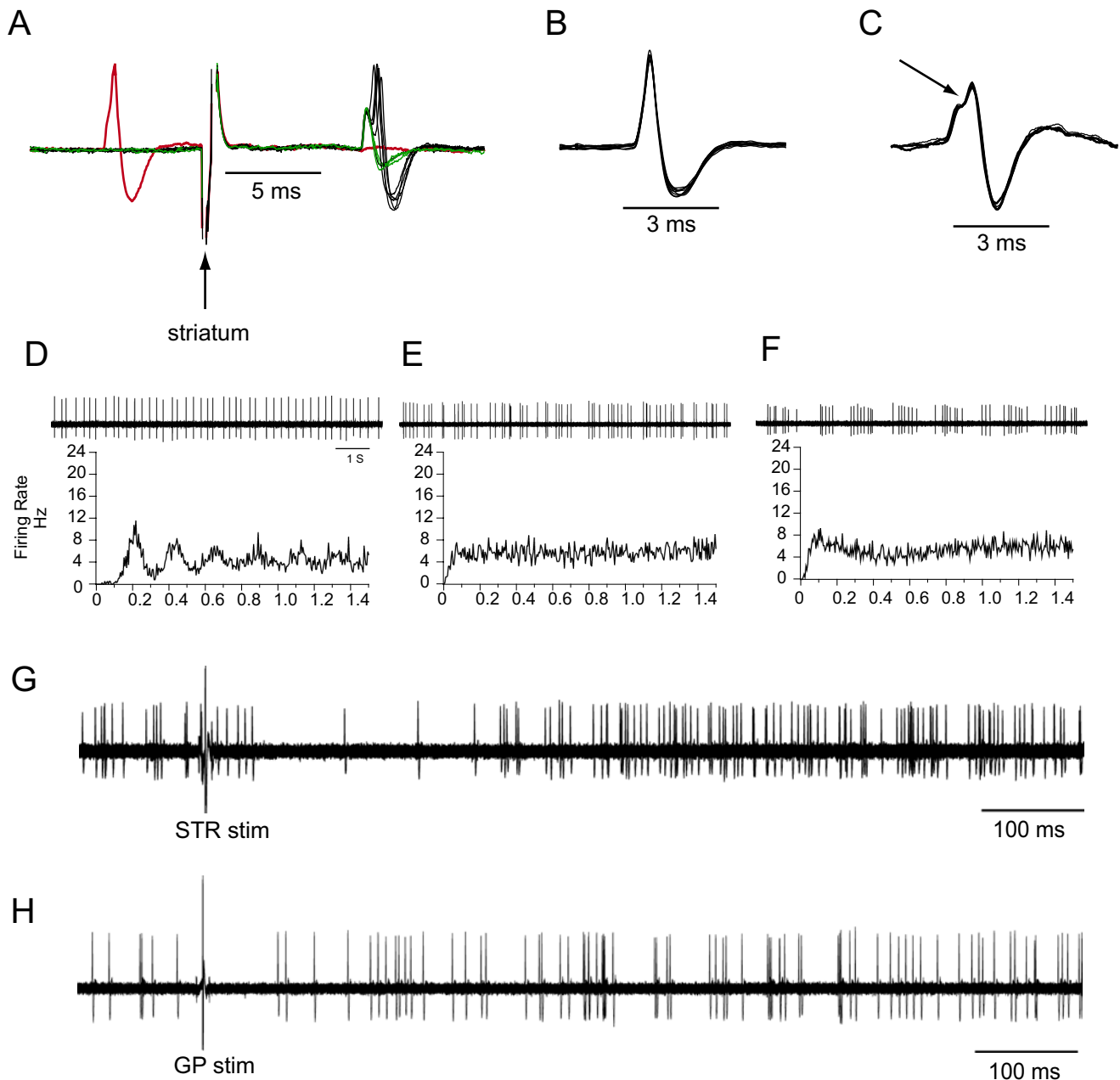
Figure 1. Representative extracellular single-unit recordings from dopaminergic neurons *in vivo* in mouse substantia nigra. *A*, Ten consecutive sweeps showing antidromic responses of a nigral neuron from striatal stimulation. Note that approximately half of the responses consist of the initial segment spike only (green traces), and that, although the initial segment latency is extremely constant, the somatodendritic component of the antidromic spike exhibits considerable latency variability (black traces). A collision occurs in the red trace. *B*, Ten consecutive spontaneous spikes (high-pass filtered) superimposed from a neuron firing in the pacemaker mode exhibit the long-duration waveform of nigrostriatal dopaminergic neurons commonly seen in rats. *C*, Five superimposed spontaneous spikes (high-pass filtered and 10 consecutive spikes averaged) from a neuron firing in the bursting mode exhibits the prominent initial segment–somatodendritic break (arrow) sometimes seen in spontaneous spikes as well as in antidromic responses. *D–F*, Samples of spontaneous activity from control neurons showing that mouse nigrostriatal neurons exhibit the same three patterns of firing seen in rat dopaminergic neurons: pacemaker (*D*), random (*E*), and bursty (*F*). Each top panel consists of 10 s of spontaneous activity with the corresponding autocorrelogram shown below. Autocorrelograms constructed from 750–100 spontaneous spikes. Bin width, 5 ms. *G*, Twenty-five consecutive sweeps superimposed to show a typical inhibitory response of a nigrostriatal neuron to single-pulse striatal stimulation (400 μA). Note the long latency to the onset of inhibition, the incomplete nature of the spike suppression, and the long-lasting inhibition. *H*, Twenty-five consecutive sweeps superimposed to show a typical inhibitory response of a nigrostriatal neuron to single-pulse stimulation of GP (500 μA). Note the rapid onset of the inhibition and the complete suppression of firing during the inhibitory period that is shorter than that evoked from striatum.

Stimulation of GP with a train of pulses produced an EIR in all 54 neurons tested with a 8.2 ± 2.4 ms onset latency and a duration of 76.6 ± 4.5 ms during which firing was reduced by 77.2 ± 2.8%, similar to the effects of single-pulse stimulation. However, in marked contrast to single-pulse stimulation, train stimulation evoked an LIR in virtually all neurons (48 of 54, 89% vs 14 of 54, 26%). The train-induced LIR exhibited a significantly longer du-

ration (295.9 ± 20.2; $p < 0.001$) and a greater depression in firing (60.3 ± 3.3%; $p < 0.001$) compared with single-pulse GP stimulation.

Responses to SNr stimulation
Thalamic stimulation selectively activates GABAergic inputs to dopaminergic neurons from the axon collaterals of pars reticulata
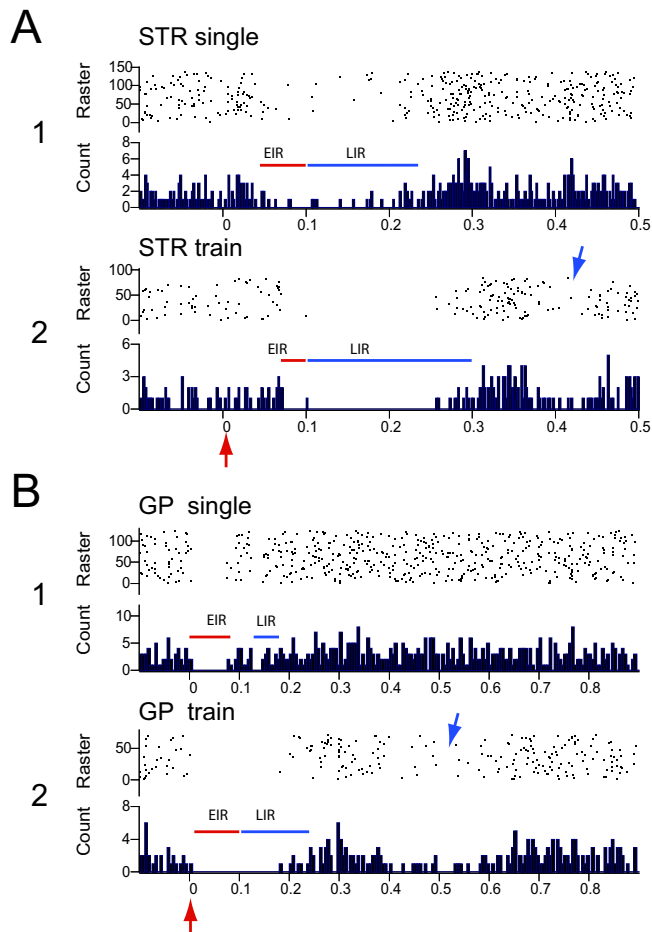
A

B

Figure 2. *A*, *B*, Effects of single-pulse stimulation and short trains of pulses (arrows) delivered to striatum (*A*; STR) and GP (*B*) on the spontaneous activity of one typical mouse nigral dopaminergic neuron *in vivo*. Note different timescales for striatal and pallidal responses. Inhibitory responses between time 0 and 100 ms were termed the early inhibitory responses (red lines), whereas those with onset >100 ms were termed the late inhibitory responses (blue lines). Note that the EIR evoked from striatal stimulation has a delayed onset and, in the case of single-pulse stimulation in *A1*, the firing is not completely suppressed. Concurrently, in both *A1* and *A2*, there is a pronounced LIR that, in the case of train stimulation, comprises complete suppression of firing. In contrast, in response to GP stimulation, the EIR exhibits a very short-onset latency and comprises complete suppression of firing. In response to single-pulse stimulation in *B1*, the LIR is brief, but, after train stimulation, the LIR is of longer duration and also comprises complete suppression of firing. Blue arrows indicate examples of a secondary, very long-latency inhibition occasionally observed. In this and all subsequent PSTHs, the stimulus is delivered at time 0 (red arrow). Each PSTH consists of 75–100 trials. Bin width, 2 ms.

projection neurons through antidromic activation of GABAergic nigrothalamic neurons in rats (Tepper et al., 1995; Paladini et al., 1999). To be certain that we could successfully antidromically activate nigrothalamic neurons in mouse, in some animals extracellular recordings were made from SNr GABAergic neurons during thalamic stimulation. We found that thalamic stimulation produced a short-latency antidromic response in seven of nine recorded pars reticulata neurons (latency, 3.7 ± 0.2 ms), similar to responses observed previously in rats (data not shown).

Also similar to results reported in rats, antidromic activation of SNr projection neurons with single-pulse stimuli produced a short-latency EIR in all tested dopaminergic neurons (*n* = 17) with a 3.8 ± 0.8 ms onset latency, a duration of 68.9 ± 6.6 ms, and a suppression of firing by 52.7 ± 7.3%. Five of 17 cells also exhibited an LIR. The mean duration of the LIR was 140.8 ± 26.3 ms, and firing during the LIR was depressed by 39.1 ± 10.7%.

Train stimulation of VT induced the EIR in all four neurons tested with an onset latency of 3.3 ± 1.7 ms and a duration of 72.5 ± 6.1 ms, during which firing rate was reduced by 41.4 ± 6.6%. All neurons also exhibited a longer duration of LIR (198.8 ± 10.1 ms) and a greater reduction in firing (58.8 ± 5.5%) to train than single pulses, although the differences did not reach statistical significance perhaps because of the small number of neurons tested.

Comparison of inhibitory responses elicited by different stimulating sites

ANOVA revealed a significant effect of stimulating site on the onset latency ($F_{(2,154)}$ = 35.7; p < 0.0001), duration ($F_{(2,154)}$ = 4.71; p < 0.02), and percentage inhibition ($F_{(2,154)}$ = 17.00; p < 0.0001) of the EIR. Subsequent *post hoc* tests showed that this was attributable to significantly shorter latencies for EIR inhibition evoked from GP (5.1 ± 0.7 ms; t = 7.82; p < 0.001) and VT (3.5 ± 0.8; t = 5.52; p < 0.001) than for those evoked from striatum (24.7 ± 2.6 ms). The EIR duration was significantly longer (t = 3.0; p < 0.01) for GP stimulation (79.1 ± 2.3 ms) than for striatal stimulation (68.1 + 3.1 ms) but did not differ significantly from that for VT stimulation (68.9 ± 6.2). The strength of the EIR evoked by GP stimulation (79.1 ± 2.3% depression) was also significantly greater (t = 5.3; p < 0.001) than that evoked by striatal stimulation (57.0 ± 3. 4%) but did not differ from that evoked by antidromic activation of SNr neurons (52.7 ± 7.1%). The stimulus site did not significantly affect the LIR.

Comparison of inhibitory responses elicited by different stimulating protocols

Comparison of single-pulse stimulation versus train stimulation pooled across stimulating sites revealed that train stimulation produced greater percentage inhibition than single pulses ($F_{(1,47)}$ = 21.24; p < 0.0001) and longer duration of inhibition ($F_{(1,47)}$ = 14.94; p < 0.0004) of the LIR but had no significant effect on either parameter of the EIR. For striatal stimulation, Bonferroni's *post hoc* analysis showed that there was a significant stimulus protocol difference for both duration of the LIR (t = 3.652; p < 0.001) and percentage inhibition (t = 2.67; p < 0.05). For GP stimulation, the two stimulus protocols also significantly differed for both percentage inhibition (t = 6.44; p < 0.001) and duration (t = 6.23; p < 0.001) of the LIR.

Effect of local GABA$_A$ blockade on inhibitory responses to striatal, pallidal, and thalamic activation

Blockade of GABA$_A$ receptors with the GABA$_A$ antagonist picrotoxin attenuated or completely eliminated the EIR induced by stimulation of all three afferent inputs as shown for three representative neurons in Figure 4 and for the population as a whole in Figure 5. ANOVA showed that local application of picrotoxin significantly attenuated both the percentage ($F_{(1,68)}$ = 117.6; p < 0.0001) and the duration of the inhibition ($F_{(1,68)}$ = 163.4; p < 0.0001) of the EIR evoked by single-pulse and train stimulation of STR, GP, or SNr but had no effect on the LIR as shown in Figure 5. The reduction or elimination of the suppression in firing rate and the shortening of the duration of the inhibition by picrotoxin was statistically significant for all individual comparisons of the EIR elicited by single-pulse or train stimulation of striatum, GP, or SNr (t = 3.35–12.02; p < 0.01–0.001). The short-latency excitation seen in Figure 4, *A* and *B*, after picrotoxin is typical and probably reflects the effects of monosynaptic and/or polysynaptic glutamatergic afferents excited by striatal or GP stimuli whose effects are usually masked by potent GABAergic inhibition when
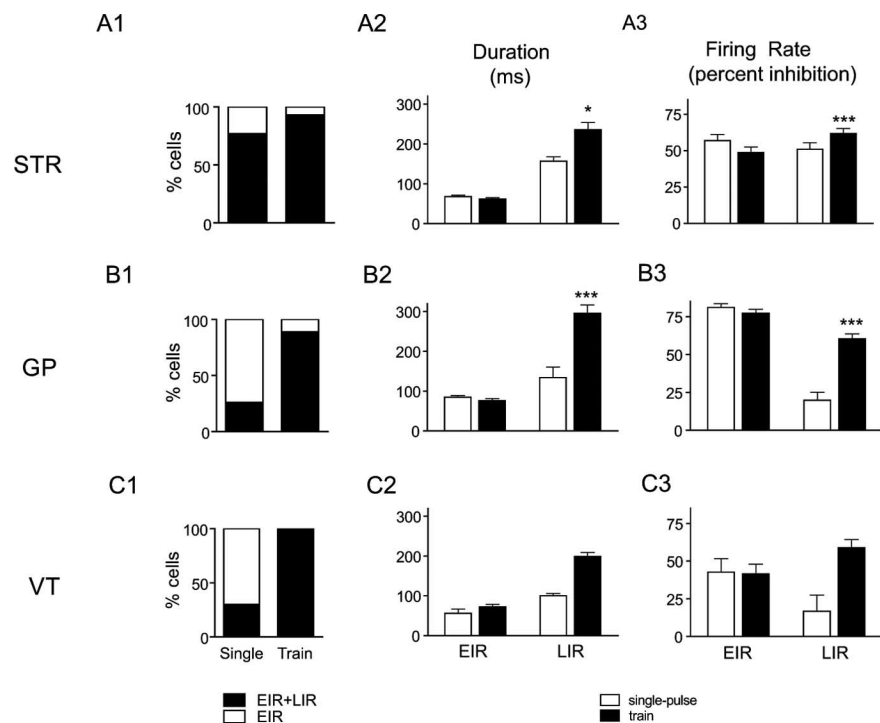
Figure 3. Summary graphs of the EIR and LIR of dopaminergic neurons to single-pulse and train stimulation of the striatum (STR), GP, and SNr. *A*, Both single-pulse and train stimulation of striatum typically evoked the EIR and the LIR, with a small proportion of cells responding with the EIR only. Train stimulation significantly increased the duration and the strength of the LIR compared with single stimuli. *B*, More than 70% of the neurons responded to single-pulse GP stimuli with EIR-only responses, whereas train stimulation elicited both the EIR and the LIR. Train stimulation in GP is extraordinarily effective at increasing the duration and strength of the evoked inhibition. *C*, Stimulation of SNr has a profile similar to that of GP in which a large proportion of neurons respond to single-pulse stimuli only with EIR. However, train stimulation evoked the LIR in 100% of the small sample of neurons tested. Train effects on duration and strength of inhibition were not significantly different from single pulse, probably because of the small *n*. *$p < 0.05$ and ***$p < 0.001$ compared with single pulse. STR, $n = 37$ neuron; GP, $n = 44$ neurons; and SNr, $n = 3$ neurons. VT, Ventral thalamus.

GABA_A receptors are not blocked (Tepper et al., 1995; Paladini et al., 1999).

The very long-latency inhibition is also apparent in Figure 4*A1* (double blue arrows) and can be seen to be abolished by subsequent picrotoxin application along with the EIR, whereas the LIR remains unaffected. We suspect that this is some kind of network-level phenomenon because the very long-onset latency and GABA_A mediation would rule out a monosynaptic response. The origin could not be determined in the present studies, and this response is not considered further in this report.

Similar to effects observed in rats after local application of bicuculline (Tepper et al., 1995; Paladini et al., 1999), in a number of neurons, application of picrotoxin unmasked a short-latency excitatory response to striatal, pallidal, or thalamic stimulation that overlapped and replaced the EIR seen under control conditions as is evident for both neurons shown in Figure 4 (single red arrows in *A* and *B*). Furthermore, the repeated secondary episodes of inhibitions that were often induced by single-pulse/or train stimulation (Fig. 2*B*, 4*A*) were also eliminated by picrotoxin administration (Fig. 4*A1,A2*). In summary, local blockade of GABA_A receptors with picrotoxin predominantly affected the EIR but has only a minimal effect on the LIR.

Effect of local GABA_B blockade on inhibitory responses to striatal, pallidal, and thalamic activation
In marked contrast to its lack of ability to attenuate stimulus-evoked inhibition in rat dopaminergic neurons, local application

of CGP55845A produced a clear attenuation of the LIR evoked by stimulation of striatum, GP, and SNr in mice. As shown previously, brief trains of stimuli significantly increased the amplitude and duration of the LIR as well as the number of neurons exhibiting the LIR to GP or SNr stimulation (up to 89%). The LIR was significantly and selectively attenuated by local application of CGP55845A. An ANOVA confirmed that duration ($F_{(1,94)} = 111.1; p < 0.0001$) and percentage inhibition of firing ($F_{(1,94)} = 163.7; p < 0.0001$) of the LIR were significantly attenuated by blockade of GABA_B receptors by CGP55845A in the majority of dopaminergic neurons. In contrast, attenuating effects on the EIR were essentially absent as shown in the population summary in Figure 6.

Presynaptic effects of GABA_B receptor blockade on inhibitory responses to striatal, pallidal, and SNr activation
In addition to attenuation of the LIR [an effect not previously observed in rats (Paladini and Tepper, 1999; Tepper et al., 1995)], local application of CGP55845A often resulted in augmentation or unmasking of the EIR as a result of blocking presynaptic inhibitory GABA_B autoreceptors on the terminals of GABAergic afferents (for recent review, see Misgeld et al., 2007), as reported previously (Paladini et al., 1999). The augmentation of inhibitory responses commonly consisted of modest increases in the strength and/or the duration of the EIR. This effect occurred predominantly in neurons that showed a relatively weak or absent EIR under predrug control conditions, as in both examples in Figure 8. CGP55845A-induced augmentation of the EIR occurred in 55% of neurons after single-pulse GP stimulation and in 36% of neurons after train GP stimulation, and in 22% of neurons after single-pulse striatal stimulation and 7% of neurons after train stimulation of striatum. Although the result was prominent in some neurons as shown in the two examples in Figure 8, statistical analysis of the entire population revealed that difference in the means between predrug condition and CGP55845A administration was not statistically significant for strength of inhibition and only of borderline significance for duration of inhibition ($F_{(1,134)} = 3.53; p = 0.058$).

Effect of local GABA_A and GABA_B receptor blockade on inhibitory responses to striatal, pallidal, and SNr activation
Local application of both picrotoxin and CGP55845A resulted in a substantial reduction or complete elimination of both the EIR and LIR induced by stimulation of STR (43 cells), GP (53 cells), or SNr (9 cells) as illustrated for two typical neurons in Figure 4 and for the entire population in Figure 7. ANOVA of data pooled across stimulus sites showed that reductions in magnitude and duration of the EIR and LIR were statistically significant (EIR percentage inhibition, $F_{(1,100)} = 123.6, p < 0.001$; LIR percentage inhibition, $F_{(1,68)} = 210.4, p < 0.001$; EIR duration, $F_{(1,100)} = 178.2, p < 0.001$; LIR duration, $F_{(1,68)} = 149.9, p < 0.001$).

The effects of $GABA_A$ and combined $GABA_A$ and $GABA_B$ receptor antagonists broken down by stimulus site and stimulation protocol shown in Figures 3 and 5–7 clearly indicate that the EIR is predominantly a result of $GABA_A$ receptor activation, whereas the LIR depends predominantly on $GABA_B$ receptor activation, independent of the stimulus site or stimulus protocol.

Effect of local $GABA_A$ or $GABA_B$ receptor blockade on spontaneous activity

As in rats (Paladini and Tepper, 1999), local application of the $GABA_A$ antagonist picrotoxin produced a significant increase in the spontaneous firing rate of mouse dopaminergic neurons from 4.6 ± 0.3 to 6.2 ± 0.3 spikes/s ($n = 41$; $t = 5.35$; $p < 0.001$). Local CGP55845A application also produced a more modest but still statistically significant effect on spontaneous activity, causing a decrease in mean firing rates from 5.4 ± 0.3 to 4.7 ± 0.2 spikes/s ($n = 62$; $t = 3.335$; $p < 0.002$). Concurrent or sequential application of both picrotoxin and CGP55845A produced a significant increase in the spontaneous firing rate in mouse dopaminergic neurons from 5.2 ± 0.2 to 6.2 ± 0.3 spikes/s ($n = 50$; $t = 4.111$; $p < 0.001$), as illustrated in Figure 9A.

In addition, just as in rats, the most prominent effects of GABAergic antagonists on spontaneous activity were on the pattern rather then the rate of firing, as shown in Figure 9B. Blockade of $GABA_A$ receptors exerted a dramatic effect on the pattern of firing in most dopaminergic neurons, leading to a more than tripling of the number of cells firing in the bursty mode but greatly decreasing the incidence of random firing and nearly eliminating the pacemaker mode as shown in Figure 9 ($\chi^2 = 16.2$; $p < 0.0004$). $GABA_B$ receptor blockade by CGP55845A caused a modest but statistically significant change in the distribution of firing patterns ($\chi^2 = 7.18$; $p < 0.03$), decreasing the proportion of neurons exhibiting burst or irregular firing and increasing the proportion of neurons exhibiting regular pattern or pacemaker-like activity, whereas simultaneous blockade of both $GABA_A$ and $GABA_B$ receptors shifted the firing pattern toward bursty firing similar to the effect of $GABA_A$ blockade alone but not quite as strongly, because the proportion of cells that shifted to the bursty mode was only 48% compared with >60% after picrotoxin alone, as shown in Figure 9B.

Effects of GABA uptake blockers

It has been suggested previously that activation of postsynaptic $GABA_B$ receptors in substantia nigra and elsewhere may require greater GABA synaptic concentrations than activation of $GABA_A$ receptors (Fritschy et al., 1999; Paladini et al., 1999; Isaacson, 2000; Scanziani, 2000) because of their preferential localization at extrasynaptic sites (Boyes and Bolam, 2003). Blocking GABA uptake by the GABA transporter (GAT) ought to increase synaptic
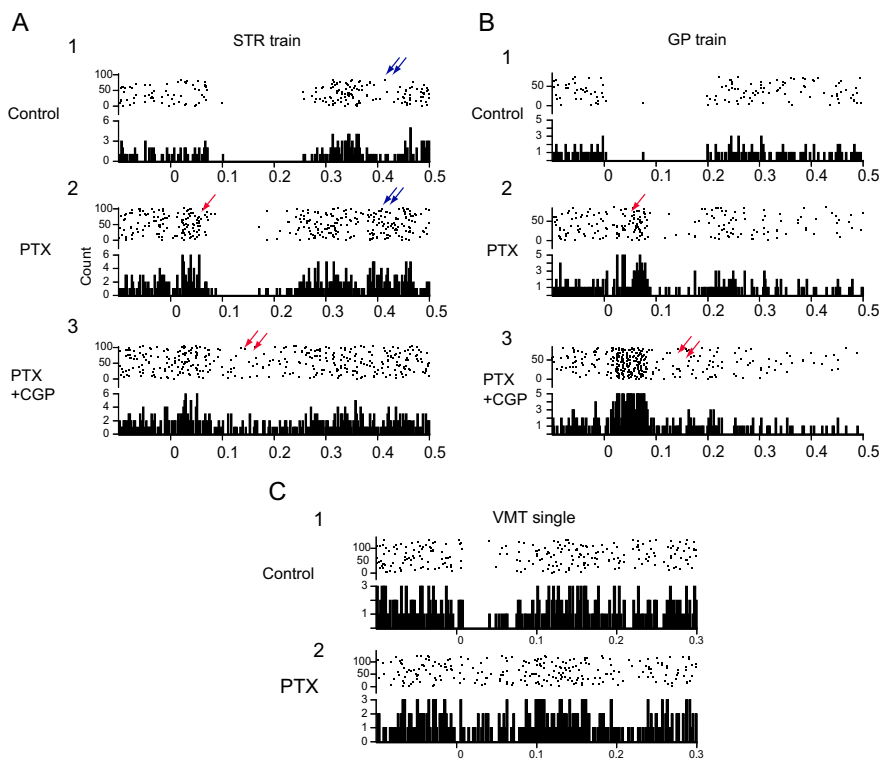

Figure 4. Typical PSTHs illustrating the effects of local blockade of $GABA_A$ and/or $GABA_B$ receptors on stimulation-induced inhibition. **A1**, Striatal train stimulation elicits an EIR and an LIR. Note delayed period of inhibition at ~400 ms (double blue arrows). **A2**, Local application of PTX blocks the EIR, unmasking a brief excitation (red arrow; for explanation, see Results) and eliminates the delayed inhibition (double blue arrows) but does not affect the LIR. Note that the overall firing rate is increased. **A3**, Subsequent simultaneous application of PTX and CGP greatly attenuates the LIR (double red arrows), and the EIR remains blocked. Note that the overall firing rate is greater than in control but less than with PTX alone in **A2**. **B1**, A brief train to GP with same parameters as in **A** evokes near-complete suppression of firing during both the EIR and LIR. **B2**, Local application of PTX completely blocks the EIR, leaving the same excitation as seen in **A2** (red arrow) but the LIR remains intact. **B3**, Subsequent combined application of PTX and CGP eliminates both the LIR (double red arrow) and the EIR. **C1**, SNr stimulation produces a short-latency EIR exhibiting complete suppression of activity. **C2**, Application of PTX completely abolishes the EIR. Note the absence of the excitatory response that is evoked by striatal or pallidal stimulation during $GABA_A$ receptor blockade. Stimulus is delivered at time 0. Each PSTH consists of 75–100 trials. Bin width, 2 ms. VT, Ventral thalamus.

levels and spillover, thereby increasing GABA receptor activation by following activation of synaptic inputs. We tested this with local pressure application of the nonspecific GAT blocker nipecotic acid (Krogsgaard-Larsen, 1980) and the intraperitoneal application of the specific GAT-1 blocker NO-711 (Borden et al., 1994). Because there was no difference between the action of locally injected nipecotic acid and systemically introduced NO-711 on spontaneous and evoked activity of the dopaminergic neurons, the data were pooled and are reported as the effects of uptake blockade.

There was no significant difference in spontaneous firing rates under control conditions compared with those during blockade of GABA uptake (5.8 ± 0.5 vs 5.6 ± 0.5 spikes/s). However, GAT blockade had a slight tendency to regularize the firing pattern. The number of cells firing in the pacemaker mode increased from 25 to 69%, whereas the number of randomly and bursty firing neurons decreased from 54 to 16% and from 23 to 15%, respectively. Analysis with χ^2 revealed that this change was of borderline statistical significance ($p = 0.503$).

We examined the effect of GABA uptake inhibition on evoked inhibition in 27 dopaminergic neurons. GAT blockade produced only a modest increase in EIR duration to striatal stimuli (88.0 ± 10.5 vs 74.4 ± 11.3 ms; $n = 12$), and a greater reduction of firing rate in the poststimulus inhibitory period (-69.9 ± 7.7 vs
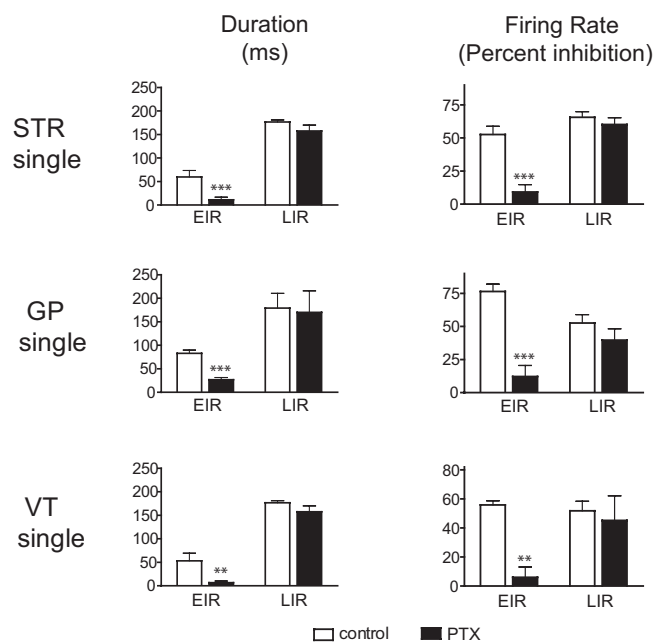
Figure 5. Summary histograms displaying the effects of local application of PTX on the duration and firing rate inhibition of the EIR and LIR evoked by single-pulse stimulation of striatum, GP, and SNr. Note that the duration of the EIR and the magnitude of the inhibition of the EIR are significantly reduced, but there is no significant effect on the LIR, indicating that the EIR is predominantly or exclusively GABA_A-mediated whereas the LIR is not. *$p < 0.05$ and ***$p < 0.001$ compared with baseline. VT, Ventral thalamus.
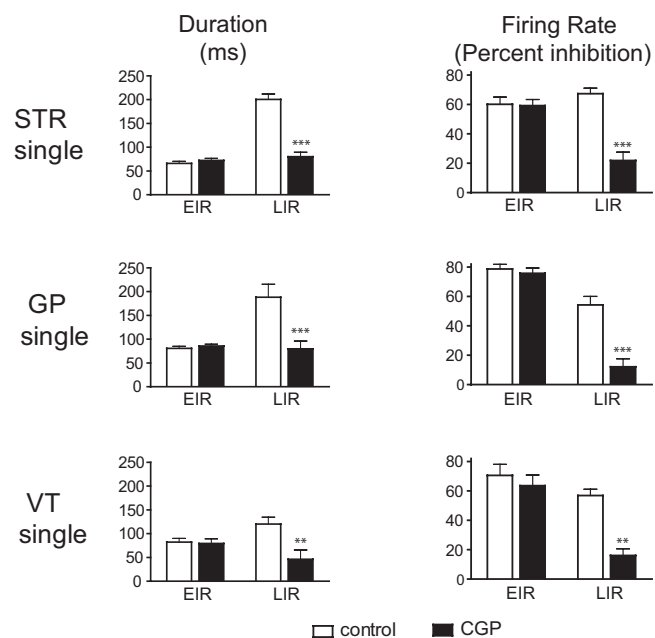

Figure 6. Summary histograms displaying the effects of local application of CGP on mean ± SEM duration and magnitude of the EIR and LIR evoked by train pulse stimulation of striatum and GP and single-pulse stimulation of SNr. Neither the duration or the magnitude of inhibition of the EIR is affected, but there is significant reduction of both duration and depression in firing rate from all three sites in the LIR, indicating that the LIR contains a significant GABA_B-mediated component whereas the EIR does not. **$p < 0.01$ and ***$p < 0.001$ compared with baseline. VT, Ventral thalamus.

$-48.3 \pm 9.5\%$; $n = 12$). With GP-evoked responses, blockade of GABA uptake had no effect on duration (94.3 ± 4.6 vs 85.7 ± 3.5; $n = 15$) or magnitude (-82.34 ± 4.62 vs 83.99 ± 4.66%; $n = 15$) of the EIR. None of these comparisons was statistically significant.

In contrast, blockade of GABA uptake significantly affected striatal- and pallidal-evoked LIR. Striatal stimuli caused a slightly stronger depression in firing rates under uptake blockade (-65.2 ± 6.2 vs $-40.9 \pm 12.2\%$; $p < 0.05$) and a significantly longer duration of inhibition (200.2 ± 21.7 vs 128.0 ± 28.7 ms; $p < 0.05$). A typical example is shown in Figure 10. For GP-evoked responses, blockade of GABA uptake produced a significantly larger magnitude of inhibition in firing in the LIR (-60.0 ± 7.3 vs 19.2 ± 8.3%; $p < 0.001$) and longer duration of inhibition (217.08 ± 2 6.64 vs 100.0 ± 24.6 ms; $p < 0.01$) as shown in Figure 10.

Some neurons that exhibited only an EIR in response to striatal or pallidal stimulation under control conditions manifest the LIR after GAT blockade. For striatal stimulation, the proportion of neurons exhibiting the LIR increased from 73 to 89%, and, after GP stimulation, the proportion of neurons exhibiting LIR increased from 31 to 80%.

To determine whether NO-711-induced augmentation of the evoked inhibition was mediated by stimulation of GABA_B receptors, we tested the ability of locally applied CGP55845A to attenuate or eliminate the effects of previous uptake blockade on electrically evoked inhibition. The GABA_B antagonist did not affect either the magnitude (-73.1 ± 6.8 vs $-79.9 \pm 4.6\%$) or the duration (92.1 ± 8.2 vs 95.3 ± 6.8 ms) of the striatal- or GP-evoked EIR under uptake blockade. In contrast, application of CGP-55845 significantly reduced the strength ($F_{(1,26)} = 76.9$; $p < 0.0001$) and the duration ($F_{(1,26)} = 109.1$; $p < 0.0001$) of the LIR evoked from striatal and GP. The GABA_B antagonist reduced the magnitude of the striatal-evoked LIR (from -65.2 ± 6.2 to $-19.05 \pm 7.33\%$ of prestimulus firing; $p < 0.001$) and also decreased the duration of the inhibition from 200.2 ± 21.7 to 57.67 ± 10.83 ms; $p < 0.001$). Identical effects were seen for the GP-induced LIR in which GABA_B blockade reduced the magnitude of inhibition from -60.0 ± 7.3 to $-7.6 \pm 7.3\%$ ($p < 0.001$) and the duration from 217.1 ± 26.6 to 16.5 ± 8.0 ms ($p < 0.01$).

Discussion

Activation of GABAergic afferents *in vivo* in mice elicits both GABA_A- and GABA_B-mediated inhibition

The principal findings in this study were as follows. First, in contrast to rats, electrical stimulation of striatum, GP, or SNr in mice consistently elicited a long-lasting inhibition that was composed of GABA_A and GABA_B components. Although very low-intensity stimulation could occasionally elicit an EIR alone, even in these cases, the EIR was longer in duration that that seen in rats. The EIR was consistently and selectively blocked by picrotoxin, whereas the LIR was unaffected by picrotoxin but was attenuated or eliminated by CGP55845A. Thus, the EIR is mediated predominantly or exclusively by activation of GABA_A receptors, whereas the LIR is mediated predominantly or exclusively by activation of GABA_B receptors. Second, blockade of GABA uptake enhanced selectively the LIR component of the evoked inhibition. Finally, analysis of the strength and duration of the evoked inhibition revealed significant differences among the inhibitory responses elicited by striatal, pallidal, or SNr stimulation.

What accounts for the expression of the GABA_B-mediated in LIR in mice but not in rats?

Single-pulse or train stimulation of striatum, GP, or SNr in mice evoked both a GABA_A-mediated EIR and a GABA_B-mediated LIR. In contrast, in previous studies in rats using even stronger stimuli, only the GABA_A-mediated EIR could be evoked (Nakamura et al., 1979; Paladini et al., 1999). The expression of the LIR

in mice is the reason for the longer-duration inhibition evoked from striatum, GP, and SNr and its GABA$_B$ sensitivity.

It is possible that different anesthetics used in this and the previous studies contributed to these differences because urethane and chloral hydrate have very different effects on GABAergic transmission (Sceniak and Maciver, 2006; Lu et al., 2008). However, this is unlikely to be the only or even the principal cause for the differences observed between the present and previous results. Most importantly, striatonigral IPSPs and/or inhibition in previous studies in chloral hydrate-anesthetized rats also appear to have been attributable to GABA$_A$-receptor stimulation (Guyenet and Aghajanian, 1978; Grace and Bunney, 1985). In addition, in a study using striatal stimulation in chloral hydrate-anesthetized rats (Dai and Tepper, 1998), we failed to note any difference between striatal-evoked inhibition of dopaminergic neurons in this preparation and in others in which we used urethane anesthesia (Tepper et al., 1990, Trent and Tepper, 1991).

A more likely possibility is the smaller size of the mouse brain. A rat brain weighs approximately five times as much as a mouse brain (Bishop and Wahlsten, 1999). The packing density of neurons in the brain varies inversely with the size of the brain (Tower, 1954) and, at least in neocortex, scales to a $-\frac{1}{3}$ power law against total cortical volume (Changizi, 2001; Harrison et al., 2002). This would cause identical amplitude electrical stimuli that stimulate equivalent volumes of tissue to activate much larger numbers of neurons and/or axons as well as a greater proportion of neuronal elements in that volume. Field stimulation of cortex *in vitro* evoked ~80% more norepinephrine release from mouse than rat slices, whereas stimulation of striatum released ~300% more dopamine from mouse than rat slices (Scholze et al., 2007), indicating that the increase in number and proportion of stimulated elements in mouse versus rat translates into similar increases in release and extracellular levels of transmitters. It is also interesting to note that striatal-evoked inhibition is of relatively long duration in the neonatal rat whose brain is similar in size to that of a mouse but decreases through postnatal development as brain size increases (Tepper et al., 1990). In mice, even single-pulse stimulation presumably results in levels of GABA in the terminal regions sufficient to allow for diffusion away from the synapse and activation of extrasynaptic GABA$_B$ receptors. This results in the recruitment of the long-latency, longer-duration GABA$_B$ response.

This explanation is further supported by the observation that train stimulation promoted the appearance of a CGP-55845-sensitive LIR, even in dopaminergic neurons that responded to single-pulse stimuli with only a brief EIR and little or no LIR
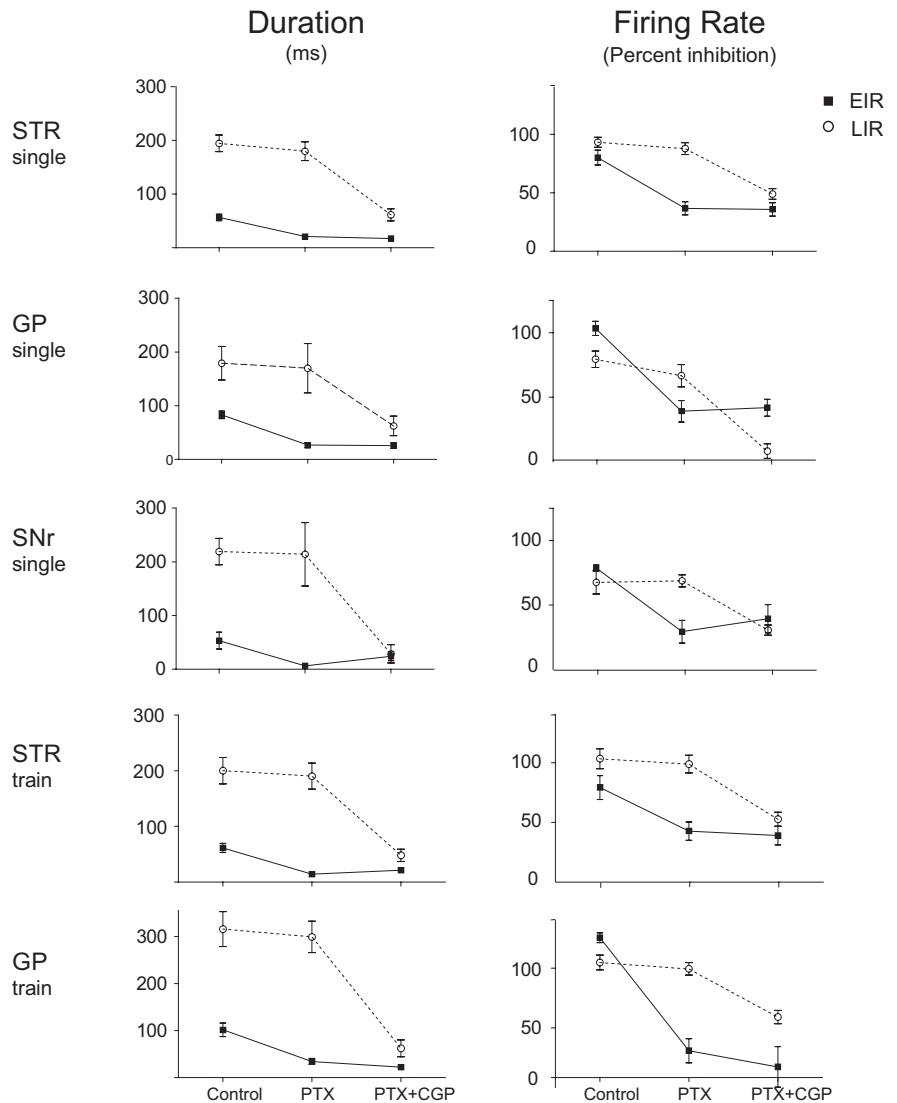

Figure 7. Summary of the effects of local application of PTX and CGP on the EIR and LIR. Means ± SEM duration and magnitude of inhibition (percentage inhibition, right) for both EIR (black circles, solid line) and LIR (white circles, dashed line) are shown for control condition (Control), after local administration of PTX, and after application of PTX together with CGP (PTX + CGP). STR, Striatum.

under control conditions. In addition, GABA$_B$ IPSP/IPSCs observed *in vitro* often requires high-frequency trains for elicitation (Lacey et al., 1988; Johnson and North, 1992; Hajós and Greenfield, 1993; Häusser and Yung, 1994; Saitoh et al., 2004). These data further support the idea that the major reason for the shorter-duration inhibitions seen in rats *in vivo* is that the stimuli evoke relatively low levels of GABA in the rat nigral terminal fields that result in predominantly or exclusively GABA$_A$ activation, whereas the same stimuli evoke higher levels of GABA in mice that are able to activate extrasynaptic GABA$_B$ receptors.

Presynaptic effects of GABA$_B$ blockade in substantia nigra

Although unable to block or attenuate the EIR, local application of CGP55845A often enhanced the strength and/or duration of the EIR, just as in rats (Paladini et al., 1999) in which this facilitation was blocked by subsequent local application of GABA$_A$ antagonists. Consistent with these findings, CGP55845A caused a slight inhibition of firing rate and a shift of firing pattern away from bursting, as it did in rats (Paladini and Tepper, 1999).
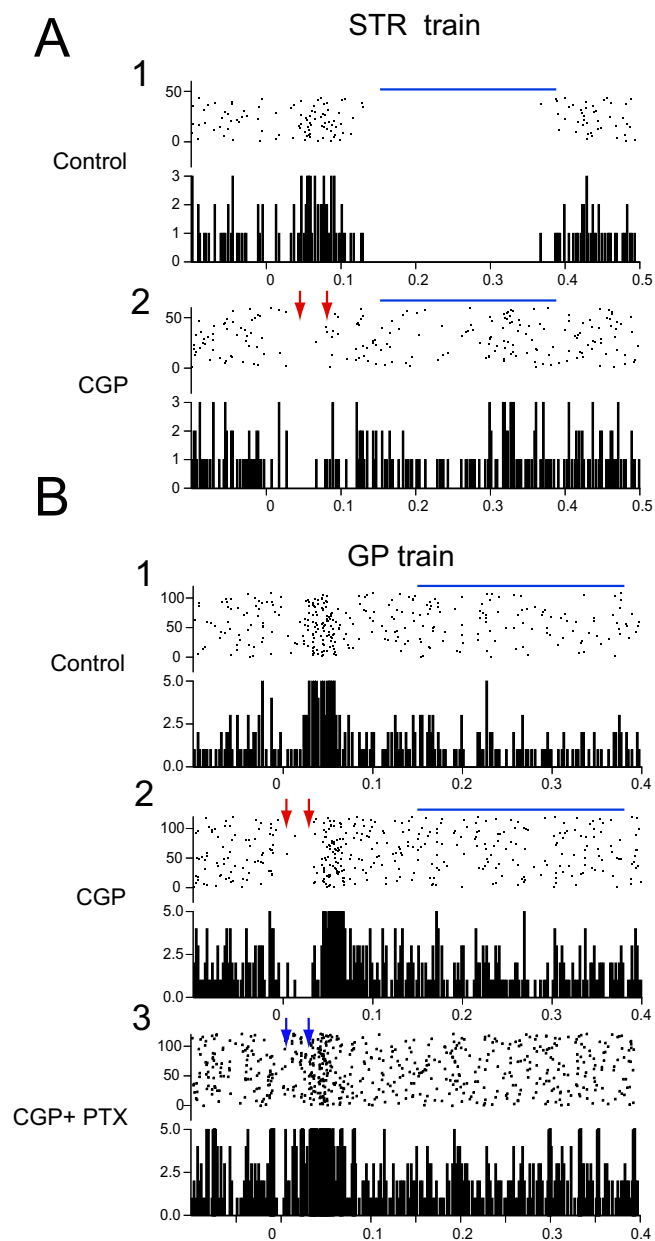
Figure 8. Presynaptic effect of GABA$_B$ receptor blockade increases EIR but blocks LIR in two representative neurons. **A1**, Control trace showing a modest EIR but a strong LIR (blue line) to striatal train stimulation. **A2**, Local application of CGP greatly increases the strength and duration of the EIR (red arrows) but at the same time almost completely abolishes the LIR (blue line). **B1**, Control trace in a second neuron showing relatively minimal EIR with incomplete suppression of activity and a more prominent LIR to GP train stimulation. **B2**, Local application of CGP greatly increases the strength and duration of the EIR, unmasking a period of complete suppression of spiking (red arrows) but also completely abolishing the LIR. **B3**, Subsequent application of both CGP and PTX abolishes the unmasked EIR (blue arrows).

These effects are almost certainly due to increased postsynaptic GABA$_A$ receptor stimulation attributable to increased release of GABA from afferents after terminal autoreceptor blockade. Striatonigral afferents to have been shown to express presynaptic GABA$_B$ receptors (Boyes and Bolam, 2003, 2007), and GABAergic IPSP/IPSCs are greatly reduced in amplitude by selective GABA$_B$ agonists in dopaminergic neurons *in vitro* (Seabrook et al., 1990; Radnikow et al., 2001; Misgeld, 2004; Saitoh et al., 2004; Misgeld et al., 2007).

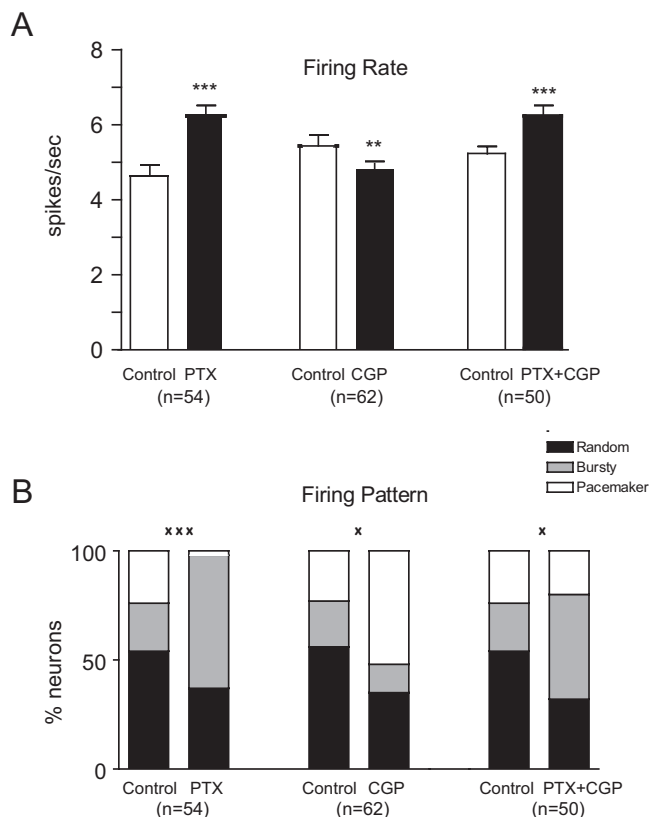Tonic activation of GABA$_A$ receptors on nigral neurons has

Figure 9. Effects of GABA$_A$ and GABA$_B$ antagonists on spontaneous activity of mouse nigral dopaminergic neurons. **A**, Under control conditions, mouse dopaminergic neurons fire spontaneously at 4.6 spikes/s, approximately the same rate as in rats. Local application of PTX significantly increased firing rate to 6.2 spikes/s. Conversely, CGP produced a modest but statistically significant decrease in firing rate from 5.4 to 4.7 spikes/s, whereas combined application of CGP and PTX was similar to the effects of PTX alone and significantly increased spontaneous firing rate from 5.2 to 6.2 spikes/s. **B**, PTX also exerts dramatic effects on firing pattern, shifting the distribution to predominantly bursty firing and essentially eliminating the pacemaker mode. CGP exerts the opposite effect, decreasing burst firing and more than doubling the proportion of neurons firing in the pacemaker mode. Combined application of PTX and CGP produces effects qualitatively similar to those of PTX alone but with smaller proportional shifts in firing pattern. Numbers below the bars indicate the number of neurons. **$p < 0.01$; ***$p < 0.001$ (Bonferroni's t test); $^{111}p < 0.001$; $^1p < 0.01$ (χ^2 test).

been inferred to occur *in vivo* and *in vitro* in rats by the powerful effects of GABA$_A$ receptor antagonists on firing rate and pattern (Rick and Lacey, 1994, Tepper et al., 1995; Paladini and Tepper, 1999; present results). In contrast, in mice, as in rats, there does not appear to be a significant GABA$_B$-mediated postsynaptic tone on the dopaminergic (Tepper et al., 1995; Paladini and Tepper, 1999; present results) or GABAergic (Rick and Lacey, 1994) nigral neurons (but see Erhardt et al., 2002), although such a tone is clearly present at the GABA$_B$ autoreceptors on the GABAergic afferents.

Effects of GABA uptake blockade on spontaneous activity and stimulus-evoked inhibition
Inhibition of GABA uptake increased the magnitude and duration of the LIR and in several cases made manifest an LIR when there was none before. Both effects were blocked by CGP55845A but not by picrotoxin, consistent with the selective blockade of the LIR by GABA$_B$ antagonists shown previously. The selective facilitation of the LIR by uptake blockade is likely attributable to increased extracellular levels of GABA allowing for greater diffusion of GABA to extrasynaptic GABA$_B$ receptors.

GABA uptake blockade also produced a very slight but statistically nonsignificant decrease in spontaneous firing rate as well as a modest increase in the proportion of neurons firing in the pacemaker mode and a concomitant decrease in the proportion of neurons firing in the bursty and random modes. These results are also very similar to the presynaptic effects of CGP55845A and are also likely attributable to a slight increase in tonic $GABA_A$ receptor stimulation. However, a direct potentiating effect of NO-711 on $GABA_A$ receptor binding is also possible (Shen and Johnson, 2001).

Differences in inhibition evoked by activation of striatal, pallidal, and nigral GABAergic afferents

In the present study, single-pulse striatal-induced inhibition was characterized by a relatively slow onset and incomplete suppression of spiking but at the same time elicited a relatively strong LIR. In contrast, activation of GP or SNr afferents produced a short-latency EIR often characterized by complete suppression of activity, yet only approximately one-third of these cells also exhibited an LIR. The LIR differences could be attributable to enriched expression of $GABA_B$ receptors in the more distal dendrites in which the majority of the striatal afferents synapse. There is also evidence that different GABAergic inputs are preferentially associated with $GABA_A$ or $GABA_B$ receptors (Sugita et al., 1992; Cameron and Williams, 1993)

The differences in onset latency are probably attributable to the pathway conduction times. The striatonigral fibers are the slowest conducting of the three main GABAergic inputs (Deniau et al., 1978; Guyenet and Aghajanian, 1978; Ryan et al., 1986; Celada et al., 1999), and the striatonigral pathway is by far the longest of the three, likely accounting for the longer onset latency from striatal stimulation compared with inhibition evoked from either GP or SNr.

The differences in strength of inhibition, conversely, may be accounted for in large part on the basis of synaptic location and number of synapses made. The majority of the striatonigral afferents synapse onto the relatively distal dopaminergic dendrites. In contrast, pallidonigral axons and inputs from SNr neurons give rise to large boutons that make a significant fraction of their contacts proximally, with individual axons making repeated contacts, sometimes forming pericellular baskets around dopaminergic somata (Smith and Bolam, 1990; von Krosigk et al., 1992; Tepper et al., 2002). These are more electrotonically favored locations and would be expected to result in more potent early inhibition compared with that evoked from striatum and also may play role in the rapid onset and typically complete suppression of firing that characterizes pallidal- and SNr-evoked inhibition. In neocortical neurons, $GABA_B$ receptor stimulation produces larger conductance increases on distal dendrites than at the soma (Takigawa and Alzheimer, 1999). A similar differential distribution of $GABA_A$ and $GABA_B$ receptors in nigral neurons could account for the delayed inhibition resulting from striatal stimulation compared with the early and potent inhibition aris-
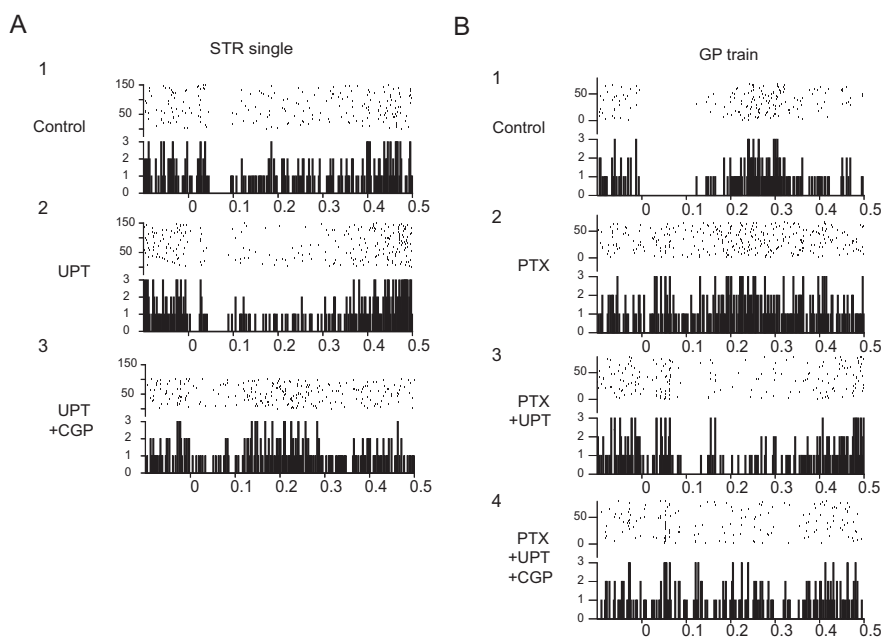

Figure 10. Blockade of GABA uptake with systemic administration of NO-711 (UPT) clearly enhances the LIR. **A1**, Baseline data show a clear EIR to single-pulse striatal stimulation with little or no LIR apparent. **A2**, After local application of UPT, the EIR is marginally enhanced, but now there is also a very clear LIR. **A3**, Combined application of UPT and CGP has a minimal attenuating effect on the EIR but completely blocks the increased LIR induced by uptake blockade. **B1**, Baseline trace showing complete suppression of firing during the EIR in response to train stimulation from GP. **B2**, Local application of PTX completely abolishes all inhibition. **B3**, Subsequent administration of NO-711 reveals a previously unseen, very pronounced LIR. **B4**, Subsequent application of CGP in the presence of PTX and GABA uptake blockade completely abolishes the LIR.

ing from GP and SNr. These differences suggest that pallidal and SNr afferents may be more important for eliciting brief, transient inhibition, whereas striatal inputs may be more important for controlling the excitability of dopaminergic neurons over longer timescales.

Conclusions

Spontaneous firing rates, firing patterns, and antidromic response properties of mouse nigrostriatal neurons are essentially identical to those characterized previously in rats. As in the rat, mouse dopaminergic neurons exhibit tonic activation of postsynaptic $GABA_A$ but not $GABA_B$ receptors under basal conditions. In marked contrast to rats, however, electrical stimulation of GABAergic inputs in mice evokes stronger and longer-duration inhibitory responses, the late component of which is $GABA_B$ mediated. The expression of the $GABA_B$ response may be attributable to the smaller size and higher neuronal packing density in the mouse brain allowing for similar stimuli to lead to much higher terminal concentrations of GABA that overflow the synapse and activate extrasynaptic $GABA_B$ receptors.

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