AMPA and GABAA receptor conductances caused the cell to go into silent firing. In contrast to NMDA receptor, application of constant GABAergic background might be expected to inhibit dopaminergic neuron firing. However, dopaminergic neurons are not all silent but instead fire in single-spiking and burst firing modes. Here we present evidence that phasic changes in the tonic activity of GABAergic afferents is a potential intrinsic mechanism that triggers bursts and pauses in dopaminergic neurons. We find that spontaneous single-spiking is more sensitive to activation of GABA receptors than phasic N-methyl-D-aspartate (NMDA)-mediated burst firing in rat slices (P15–P31). Because tonic activation of GABA_{A} receptors has previously been shown to suppress burst firing in vivo, our results suggest that the activity patterns seen in vivo are the result of a balance between excitatory and inhibitory conductances that interact with the intrinsic pacemaking currents observed in slices. Using the dynamic clamp technique, we applied balanced, constant NMDA and GABA_{A} receptor conductances into dopaminergic neurons in slices. Bursts could be produced by disinhibition (phasic removal of the GABA_{A} receptor conductance), and these bursts had a higher frequency than bursts produced by the same NMDA receptor conductance alone. Phasic increases in the GABA_{A} receptor conductance evoked pauses in firing. In contrast to NMDA receptor, application of constant AMPA and GABA_{A} receptor conductances caused the cell to go into depolarization block. These results support a bidirectional mechanism by which GABAergic inputs, in balance with NMDA receptor-mediated excitatory inputs, control the firing pattern of dopaminergic neurons.

I N T R O D U C T I O N

Dopamine released by the activity of midbrain dopaminergic neurons plays an important role in Parkinson’s disease (Bladi et al. 2000), reinforcement learning (Schultz 1998), and schizophrenia (Grace 1991). At least 70% of all inputs onto dopaminergic neurons are GABAergic, the majority of which arise from the striatum, globus pallidus (GP) and substantia nigra pars reticulata (SNr) (Bolam and Smith 1990; reviewed in Tepper and Lee 2007). Because spontaneously active neurons in the GP and SNr fire at rates as high as 50 and 60 Hz, respectively (Celada et al. 1999; Deniau et al. 1978; Guyenet and Aghajanian 1978; Kita and Kitai 1991), the activity of dopaminergic neurons is subject to a large GABAergic input. Dopaminergic neurons under the influence of this GABAergic inhibition would be expected to be mostly silent. Although some dopaminergic neurons are silent (Grace et al. 2007; but see Dai and Teppper 1998), many of them are spontaneously active in anesthetized or in awake, behaving animals (Freeman et al. 1985; Hyland et al. 2002; Kiyatkin and Rebec 1998; Schultz et al. 1997). They typically fire single spikes with varying degrees of regularity and can generate high-frequency bursts by a mechanism activating N-methyl-D-aspartate (NMDA) receptors (Chergui et al. 1993; Deister et al. 2009; Overton and Clark 1992, 1997; Zweifel et al. 2009). Dopamine is released either tonically or phasically depending whether the neuron is in a single-spiking or burst-firing mode (Goto et al. 2007; Grace and Bunney 1984a,b; Wilson et al. 1977). Does tonic GABAergic input simply hinder these firing modes or could it play a more integral role in their generation?

Dopaminergic neurons receive a combination of tonic inhibitory and excitatory inputs in vivo. Dopaminergic neurons are bombarded by chloride-mediated inhibitory postsynaptic potentials (IPSPs) in vivo (Grace and Bunney 1985). Local application of GABA_{A} receptor (GABA_{AR}) antagonists shifts the firing pattern of dopaminergic neurons from a single-spike mode into a burst-firing mode, whereas application of GABA_{B} receptor antagonists regularizes the firing pattern (Brazhnik et al. 2008; Engberg 1993; Paladini and Tepper 1999). These results were interpreted to mean that tonic activation of GABA_{A} receptors inhibits burst firing and that the action of GABA_{B} receptors is mostly presynaptic. The shift into the burst-firing mode suggests that dopaminergic neurons are also subject to tonic excitation. Local application of NMDA receptor (NMDAR) antagonists, but not AMPA receptor (AMPA) antagonist, significantly reduced burst firing (Chergui et al. 1993; Overton and Clark 1992), suggesting that the majority of the tonic excitation driving bursts is NMDAR mediated.

Here we show that spontaneous, single-spike firing is more sensitive to activation of both GABA_{A} and GABA_{B} receptors than phasic, NMDAR-mediated burst firing evoked by either iontophoresis or dynamic clamp. This suggests that the activity seen in dopaminergic neurons in vivo is the result of a balance between excitatory and inhibitory conductances. Single spiking continued after application of balanced, constant NMDAR/ GABA_{AR}, but not AMPA/GABA_{AR}, conductances by dynamic clamp. Applying tonic NMDAR and GABA_{AR} conductances, we show that phasic removal of the GABA_{AR} conductance causes burst firing and phasic increases in the GABA_{AR} conductance causes a pause in firing. Bursts generated by disinhibition have a higher frequency than bursts generated by identical excitation alone. Our results provide evidence for an extrinsic mechanism by which phasic changes in GABAergic drive can generate bursts and pauses in firing in midbrain dopaminergic neurons.
METHODS
Slice preparation and recordings

Electrophysiological experiments were performed on slices obtained from Sprague-Dawley rats (Charles River Laboratories) 15–31 days of age. Although dopaminergic neurons recorded in vivo do show changes in firing pattern with age (Tepper et al. 1990), we found no difference among neurons from 15- or 31-day-old animals during in vitro recordings. All experimental procedures were approved by the University of Texas at San Antonio Institutional Animal Care and Use Committee. Rats were anesthetized with ketamine/xylazine and decapitated, and the brains were rapidly removed and cooled. Horizontal slices (240 μm) were cut using a vibrating microtome (Microm HM 650V) in oxygenated, cold artificial cerebrospinal fluid (ACSF) containing (in mM) 110 cholineCl, 2.5 KCl, 1.25 NaH2PO4, 7 MgCl2, 0.5 CaCl2, 10 dextrose, 25 NaHCO3, 1.3 ascorbic acid, and 2.4 sodium pyruvate. Slices were transferred to an incubation chamber containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 4 MgCl2, 2 CaCl2, 10 dextrose, 25 NaHCO3, 1.3 ascorbic acid, 2.4 sodium pyruvate, and 0.05 glutathione. Slices were incubated at 32°C for at least 1 h before recording and kept at room temperature thereafter. Before recording, a slice was held in a submerged chamber filled with ACSF similar to the incubating solution except that 2 mM MgCl2 was used, and glutathione was not added. The slice was superfused at a rate of 2 ml/min by a gravity feed system and heated to 32–34°C with an inline heater.

SNc neurons were visualized with a gradient contrast imaging system. Perforated-patch or whole cell recordings were made from presumed SNc dopaminergic neurons. Perforated patch recordings were made with the whole cell internal described below or an internal solution containing (in mM) 140 KMeSO4, 0.2 EGTA, 7 NaCl, and 10 HEPES. The antibiotic gramicidin A or D (dissolved in DMSO, 100 μg/ml of internal solution) was used to maintain natural intracellular Cl− levels (Kyrozis and Reichling 1995) and to reduce run-down. Accidental break-in was determined by a large, instantaneous jump in spike height. Cells in which accidental break-in occurred were rejected. Whole cell recordings were made with an internal solution containing (in mM) 138 K-glucolone, 10 HEPES, 2 MgCl2, 0.2 EGTA, 0.0001 CaCl2, 4 Na-ATP, and 0.4 Na-GTP. All internal solutions were adjusted to a pH of 7.3 using 1 M KOH and an osmolarity of 270–275 mOsm. Recordings were acquired with a Multiclamp 700B and digitized (Instrutech) under command of the AxographX software program.

Dopaminergic neurons were identified by a slow, spontaneous firing rate (usually 1–4 Hz), a prominent spike afterhyperpolarization, and a large I1 current on passage of a hyperpolarizing voltage step. The presence of a large mGlur1-mediated hyperpolarizing response to iontophoresis of glutamate after a burst (Morikawa et al. 2003) was also used to identify dopaminergic neurons (Marino et al. 2001).

Dynamic clamp

Dynamic clamp experiments were conducted in whole cell mode as previously described (Deister et al. 2009). The equations used to calculate the applied current are

\[ I_{\text{NMDA}} = -g_{\text{NMDA}} \times \left( \frac{1}{\left\{1 + (\frac{[\text{Mg}]}{3.57}) \times e^{(-V_m - 0.621) / 200}\right\}} \times (V_m - E_{\text{NMDA}}) \right) \]

\[ I_{\text{GABA}} = -g_{\text{GABA}} \times (V_m - E_{\text{GABA}}) \]

\[ I_{\text{GABA}} = -g_{\text{GABA}} \times (V_m - E_{\text{GABA}}) \]

where [Mg] = 1.5 mM, \( E_{\text{NMDA}} = 0 \) mV, \( E_{\text{GABA}} = -60 \) mV (−63 mV was the mean reversal potential for GABA_A receptors in perforated patch; Gulăscă et al. 2003), and \( E_{\text{GABA}} = -100 \) mV unless otherwise stated. An AMPAR-mediated current was created from the \( I_{\text{NMDA}} \) equation by setting [Mg] to 0. All recordings were done with a balanced bridge in continuous current clamp (Bridge Mode). A junction potential of −6 mV was corrected on-line in these experiments.

Evoked responses

Iontophoresis was chosen over electrical stimulation of the slice to evoke phasic bursts that did not contain a GABA-mediated component, which would be concomitantly activated on electrical stimulation. Iontophoresis was also chosen over bath application of NMDA (Komendantov et al. 2004; Paladini et al. 1999b) to isolate the effects of GABA receptor activation on single-spiking and burst firing. Bursts were generated by iontophoresis of glutamate (typically onto dendrites 50 μm from the recording electrode; 50–250 ms pulses, holding +1 to 10 nA, ejection −50 to −300 nA; Dagan ION-100) as previously described (Deister et al. 2009). The iontophoretic pipette contained 1 M glutamate at pH 7–9. All experiments involving iontophoresis were performed in the presence of the AMPA receptor blockers, NBQX, or GYKI-52466.

Drugs

All drugs were applied to the slice via superfusion. Isoguvacine hydrochloride (1–100 μM), (R)-baclofen (0.01–10 μM), picrotoxin (100 μM), NBQX (25 μM), GYKI-52466 (20–50 μM), and CGP-55845 (1–2 μM) were purchased from Tocris.

Data analysis

Action potentials were detected using a derivative threshold in AxographX (1–5 V/s), and changes in spiking frequency were measured. Analysis was done with Mathematica 7 (Wolfram Research). A burst was defined as a series of the action potentials that occurred within 1 s after the onset of the iontophoretic pulse. Bursts evoked by dynamic clamp were analyzed for the entire time window that the NMDA conductance was on. In the case of bursts evoked by disinhibition, the window was defined as the period in which the GABA_A conductance was physically turned off. Maximum burst frequency was determined as the reciprocal of the minimum interspike interval (ISI) for all spikes in the burst. Mean burst frequency was determined as the reciprocal of the mean intraburst ISI. For spontaneous single spiking, mean frequency was determined as the reciprocal of the mean ISI measured over a time window of 20 s in which no current was injected to the cell. Average membrane potential was calculated as the mean of all sampled voltages, including spikes.

Statistics

In several experiments, inhibition of single-spike or burst firing frequency by GABA receptor activation was measured. Because one of the factors (GABA_A activation) was quantitative rather than categorical, ANOVA was not appropriate. Instead, regression analysis was used to fit each curve. The data were fit with a quadratic model. This was implemented in SAS (SAS Institute) using the equation

\[ y_i = \tau_1 + \beta_1 x_i + (\tau\beta_2)x_i + \beta_2 x_i^2 + (\tau\beta_2)x_i^2 + e \]

where \( y_i \) = dependent variable representing inhibited firing frequency in terms of percent of control, \( \tau \) = effect of firing frequency type (Figs. 1 and 2, B and E: maximum and mean burst frequency and mean single spiking frequency) or receptor type (Fig. 2D: GABA_A and GABA_B), \( \beta_1, \beta_2, (\tau\beta_2), (\tau\beta_2) \) = regression coefficients, \( x \) = continuous independent variable for GABA_A activation in terms of concentration of agonist (μM) or conductance applied (nS), and \( e \) = residuals, \( e \sim N(0,\sigma^2) \).

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For dynamic clamp experiments in which a range of GABA\textsubscript{A}R conductances were applied to the same cell, an additional random effect $c_k$ was added to $y_i$ to account for within-cell effects.

After each curve was fit, multiple comparisons of firing or receptor effect $y_i$ given GABA\textsubscript{A}R conductance were made using the Scheffe adjustment ($P < 0.05$). These comparisons permit us to compare, for example, whether the inhibition of single-spike firing was statistically different from the inhibition of burst firing at a specific GABA\textsubscript{A}R conductance.

For all other statistical tests, Prism (Graphpad Software) was used. All effects are given in terms of means $\pm$ SE unless stated otherwise. In all experiments presented here, statistical significance is considered at $P < 0.05$.

**RESULTS**

**GABA\textsubscript{A} receptor activation**

We first studied the inhibition of spontaneous single-spiking and NMDAR-mediated burst firing by activation of GABA\textsubscript{A} receptors. Identified dopaminergic cells fired spontaneously (typically 1–4 Hz) in a regular, single-spike firing pattern during perforated patch recordings. Bursts of action potentials (mean, 22 Hz maximum burst frequency; similar to in vivo, e.g., Grace and Bunney 1984a) were evoked every 30 s by iontophoretic application of glutamate onto the recorded neuron in the presence of an AMPA receptor antagonist, NBQX (25 $\mu$M), or GYKI-52466 (20–50 $\mu$M). We applied the GABA\textsubscript{A}R agonist isoguvacine (1, 10, 40, or 100 $\mu$M), to the bath and measured its effect on single-spike and burst firing frequency (Fig. 1). Regression analysis of the concentration-response data showed that isoguvacine inhibited single-spike firing at significantly lower concentrations than burst firing ($P < 0.05$, 15–75 $\mu$M isoguvacine, regression analysis). Application of 100 $\mu$M isoguvacine abolished both single-spike and burst firing (Fig. 1B). The addition of the GABA\textsubscript{A}R antagonist, picrotoxin (100 $\mu$M), reversed the effects of isoguvacine (Fig. 1A; $P < 0.05$, repeated-measures ANOVA with Tukey’s multiple comparison test; control single-spike firing frequency: $2 \pm 0.57$ Hz, 40 $\mu$M isoguvacine: $0 \pm 0$ Hz, picrotoxin: $1.9 \pm 0.68$ Hz, $n = 5$). Spontaneous and burst firing frequencies in the presence of picrotoxin were not significantly different from control ($P > 0.05$; repeated-measures ANOVA with Tukey’s multiple comparison test). These results suggest that spontaneous, single-spiking is more sensitive to GABA\textsubscript{A}R activation than burst firing.

We obtained similar results by applying a range of GABA\textsubscript{A}R conductances (0–30 nS) into cells using dynamic clamp (Fig. 1, C and D; Robinson and Kawai 1993; Sharp et al. 1993). Bursts (20.6 Hz maximum burst frequency on average)
were evoked by application of a moderate NMDAR conductance (35 nS; range, 20–40 nS). These conductances were high enough to cause sustained high-frequency firing but were generally not high enough to cause the cell to go into a state of depolarization block by the end of the conductance pulse (which typically occurred at conductances >60 nS; data not shown). Again, single-spiking was more sensitive to GABAAR activation than burst firing ($P < 0.05, 0–24$ nS, regression analysis). Both single-spike and burst firing were abolished in all cells at a GABAAR conductance of 30 nS. The average GABAAR conductance at which single-spike firing was suppressed was 25% of the conductance needed to suppress burst firing (Fig. 1D; single-spike 4.8 ± 2.1 nS; burst, 19.5 ± 4.5 nS; $n = 4$). Together, these results show that in the presence of a tonic GABAAR conductance, single-spike firing is more sensitive to GABAAR activation than burst firing.

**GABA$_B$ receptor activation**

Tonic activity in GABAergic afferents may also activate postsynaptic GABA$_B$ receptors on midbrain dopaminergic neurons. Cells were recorded in perforated patch and NMDAR-mediated bursting was evoked by iontophoresis at regular intervals as above. The GABA$_B$R receptor agonist, baclofen (0.01, 0.1, 1, or 10 $\mu$M), suppressed single-spike and burst firing in a dose-dependent manner (Fig. 2). Single-spike firing was inhibited at lower concentrations than burst firing ($P < 0.05, 0.6–2$ $\mu$M baclofen, regression analysis).

Inhibition of single-spike and burst firing by application of a GABA$_B$R conductance was also investigated in dynamic clamp (Fig. 2, C and D; $E_{\text{GABA}_B} = -100$ mV). Bursts were evoked by application of a NMDAR conductance as before (Fig. 1C–D). There was a significant difference in the regressions of the single-spike inhibition curves and both of the burst firing inhibition curves ($P < 0.05, 0–8$ nS, regression analysis).

We also tested whether GABA$_B$R activation was more effective at suppressing firing than GABAAR activation. The use of dynamic clamp allowed for direct comparison of these two receptor types in the same sample (from Fig. 1D). We found that there was a significant difference between GABAAR and GABA$_B$R conductances for inhibition of burst firing ($P < 0.05, 0–24$ nS, regression analysis) and single-spike firing ($P < 0.05, 0–7.2$ nS, regression analysis).

Together these results extend our findings with GABAAR to show that with activation of either GABAAR or GABA$_B$R receptors single-spike firing will be abolished before the suppression of burst firing mediated by the activation of an NMDAR conductance. These results further show that GABA$_B$R receptors are more effective at suppressing both single-spike and burst firing than GABAAR. Because the conductances used in Fig. 2D were equal, this indicates that GABA$_B$R are more effective because of the more hyperpolarized reversal potential for potassium.

**Bursting by disinhibition**

Previous studies have suggested that bursts are suppressed by tonic activation of GABA$_A$R in vivo (Brazhnik et al. 2008; Brazhnik et al. 2008). However, it is not clear how this occurs. Tonic GABAergic activity may also activate GABA$_B$ receptors postsynaptically. Therefore, we also investigated the ability of disinhibition to suppress bursting activity by application of a GABA$_B$R conductance (Brazhnik et al. 2008).

**FIG. 2.** Single-spike firing is more sensitive to GABA$_B$R activation than NMDAR-mediated burst firing. A: a phasic burst was evoked in a perforated patch recording of an identified dopaminergic neuron by iontophoresis of glutamate (Glu; black bars beneath trace; top) in 25 $\mu$M NBQX. Bath application of 1 $\mu$M baclofen abolished single-spike activity and reduced the burst firing frequency. This effect was reversible on removal of baclofen [return to ACSF]. Spike heights are truncated. B: summarized data for the percent of inhibition of maximum burst frequency (filled circles), mean burst frequency (gray circles), and mean spontaneous firing frequency (empty circles) from control after bath application of baclofen (0.01, 0.1, 1, and 10 $\mu$M). Numbers in parentheses represent number of cells. C: a representative recording showing inhibition of single-spike and burst firing by application of a 1.2 nS GABA$_B$R conductance ($E_{\text{GABA}_B} = -100$ mV). The total current (truncated) applied by the dynamic clamp is shown above each voltage trace. D: summarized data ($n = 4$) show the effectiveness of GABA$_A$R- and GABA$_B$R-mediated inhibition in suppressing maximum burst frequency (filled squares and circles, respectively) and mean spontaneous firing frequency (empty squares and circles, respectively). Conductances were applied into the same group of cells.
Paladini and Tepper 1999). However, here we show that single-spike firing is more sensitive than burst firing to activation of either GABA_A or GABA_B receptors (Figs. 1 and 2). Therefore tonic activation of GABA receptors in vivo would be expected to suppress single spiking, causing dopaminergic neurons to be silent. However, single spiking is seen in vivo (Grace and Bunney 1984b; Paladini and Tepper 1999; Wilson et al. 1977). This suggests that the firing pattern recorded in vivo is caused by a combination of excitatory and inhibitory conductances in addition to its pacemaking currents. These results also suggest that phasic removal of this inhibitory conductance can evoke a burst and that a phasic increase in this inhibitory conductance can evoke a pause in firing.

A control burst was evoked by application of a constant NMDAR conductance in whole cell recordings, as shown above (Figs. 1C and 2C). The same NMDAR conductance was applied in the presence of a constant GABA_A conductance ($E_{GABA_A} = -60$ mV; $-63$ mV measured in perforated patch recordings in Gulácsi et al. 2003) sufficient to counteract the NMDAR conductance-mediated burst and return single-spike firing back to control levels (Fig. 3, A and B; $P > 0.05$; control mean ISI, 0.55 ± 0.11 s; balanced ISI, 0.67 ± 0.18 s; paired $t$-test, $n = 10$). Overall, the ratio of NMDAR/GABA_A conductance used was 3.2 ± 0.4 ($n = 7$). In similar experiments using an AMPAR conductance instead of NMDAR, background firing could not be restored. Instead, the cell quickly went into depolarization block (Fig. 3D; 4/4 cells). Block in the AMPA/GABA_A configuration occurred at much smaller conductances than those used in the balanced NMDAR/GABA_A configuration (typical NMDAR/GABA_A conductance: 25/8 nS; AMPA/GABA_A conductances were <4 nS each).

In the NMDAR/GABA_A balanced configuration, a burst of action potentials could be evoked by briefly turning off the GABA_A conductance (Fig. 3A). A pause in firing was evoked by transiently turning off the NMDAR conductance (Fig. 3A) or by increasing the GABA_A conductance (Fig. 3C). Bursts produced by disinhibition had a greater frequency than control bursts with an identical NMDAR conductance (Fig. 4, A and B; $P < 0.05$; control maximum burst frequency, 22.2 ± 4.9 Hz; disinhibition, 27.8 ± 5.4 Hz; paired $t$-test, $n = 10$; 1st 5 spikes mean burst frequency; control, 13.8 ± 1.6 Hz; disinhibition, 20.0 ± 3.4 Hz; paired $t$-test, $n = 10$). Both disinhibition and excitation-only bursts displayed prominent spike frequency adaptation (Fig. 4A). The increase in burst firing frequency was sustained over the first eight spikes of the burst (Fig. 4C). This suggests that the mechanism by which disinhibition bursts are faster occurs on the order of hundreds of milliseconds.

The mechanism by which disinhibition bursts are faster than control bursts may lie in differences in the availability of spiking currents just before the removal of the GABA conductance (Fig. 5). To test this, we constructed phase plots of both spontaneous single-spike (Fig. 5A, black) and balanced conductance single-spike modes (Fig. 5A, red). There was a significant decrease in maximum $dV_{m}/dt$ (Fig. 5B; $P < 0.05$; control 67.8 ± 8.3, balanced 44.4 ± 2.9, paired $t$-test, $n = 7$). There was also a significant increase in minimum $dV_{m}/dt$ (Fig. 5B; $P < 0.05$; control −41.0 ± 4.9, balanced −29.7 ± 3.6, paired $t$-test, $n = 7$). Balanced spiking had a more depolarized threshold than control spiking (Fig. 5B; $P < 0.05$; control −34.4 ± 1.3 mV, balanced −32.0 ± 1.5 mV, paired $t$-test, $n = 7$). This suggests that an increase in sodium channel availability is not the cause of the increased burst frequency of disinhibition bursts.

Histograms of control and balanced spiking showed that the average membrane potential was significantly more depolarized for balanced than control spiking (Fig. 5, C and D, also Fig. 5A; example histogram taken from cell shown in Fig. 4A; $P < 0.05$; control −46 ± 1.0 mV, balanced −39 ± 1.5 ms, paired $t$-test, $n = 10$). There was also a significant decrease in the latency from the time of conductance change to the peak of

![Fig. 3.](http://jn.physiology.org/)

**Fig. 3.** Bursts and pauses can be generated by phasic changes in input. A: in a whole cell recording, balanced GABA_A and NMDAR conductances were applied into a dopaminergic neuron. A disinhibition burst could be evoked by phasic removal of the GABA_A conductance. A pause in firing could be evoked by phasic removal of the NMDAR conductance (A) or by a phasic increase in the GABA_A conductance (C; increase of 2 nS). The total current applied by the dynamic clamp is shown above each voltage trace. The total current in C has been truncated. B: single-spike frequency in the presence of tonic NMDAR and GABA_A (Balanced) conductances was not significantly different from spontaneous, single-spike firing (Control; $P > 0.05$, paired $t$-test). Horizontal lines indicate the means for the sample. D: single spiking is not maintained with application of balanced GABA_A and AMPAR conductances.
the first action potential in the burst (Fig. 5E; \( P < 0.05 \); control 58 \( \pm \) 16 ms, disinhibition 27 \( \pm \) 9 ms, paired \( t \)-test, \( n = 10 \)). This suggests that a current that inactivates with depolarization (e.g., an A-type potassium current) may be the cause of the increased burst frequency of disinhibition bursts.

**DISCUSSION**

**High conductance state of the dopaminergic neuron**

Dopaminergic neurons recorded in vivo display a variety of firing patterns: silent, regular single-spiking, irregular single-spiking, and bursty (reviewed in Tepper and Lee 2007). Dopaminergic neurons recorded in slices, however, fire only in the regular, single-spiking mode. This difference in firing patterns of dopaminergic neurons recorded in slices and in vivo is often ascribed to the loss of afferent input in slices (Overton and Clark 1997). This suggests that single-spiking in vivo is generated by an in vitro–like pacemaking mechanism in which spikes can be advanced or delayed because of afferent input (hereafter referred to as the pacemaker mechanism). Under such a scheme, bursts are caused by phasic excitatory inputs and pauses in firing are caused by phasic inhibitory inputs. Such a mechanism may underlie the reward-related responses of dopaminergic neurons described by Schultz (1998). However, additional mechanisms for generating phasic bursts and pauses are likely for dopaminergic neurons in the high conductance state (reviewed in Destexhe et al. 2003).

There is much evidence that suggests the presence of a tonic inhibitory drive onto dopaminergic neurons. Dopaminergic neurons are bombarded by chloride-mediated IPSPs in vivo (Grace and Bunney 1985). These IPSPs are most likely caused by spontaneously active afferent cells in the globus pallidus (GP) or substantia nigra pars reticulata (SNr), which fire at \( \sim \)50 Hz (Celada et al. 1999; Deniau et al. 1978; Guyenet and Aghajanian 1978; Kita and Kitai 1991). The presence of this tonic inhibitory drive can also be shown by the local application of GABA antagonists in vivo. Paladini and Tepper (1999); and Brazhnik et al. (2008) found that local application of a variety of GABA\(_A\) antagonists by pressure ejection onto the recorded neuron shifted the firing pattern of the dopaminergic neuron from a single-spiking mode to a bursting one, indicating that tonic GABA\(_A\)R activation suppresses burst firing.

Our results, showing that NMDA-mediated burst firing is suppressed at greater levels of both GABA\(_A\) and GABA\(_B\) receptor activation than single spiking, also suggest that dopaminergic neurons are tonically inhibited in vivo. However, if single spiking in vivo is generated by a pacemaker mechanism, our results suggest that both single-spiking and bursting would be suppressed and there would be no tonic level of dopamine in efferent structures. However, many dopaminergic neurons recorded in vivo are not silent (Grace and Bunney 1984a,b; Paladini and Tepper 1999; Paladini et al. 1999a; Wilson et al. 1977), and tonic levels of dopamine are observed at target loci (Floresco et al. 2003; Gonon 1988). Therefore our results suggest that dopaminergic neurons receive tonic excitatory input.

Local application of NMDAR antagonists but not an AMPAR antagonist significantly reduce spontaneous burst firing (Chergui et al. 1993; Overton and Clark 1992; see also Charlety et al. 1991), suggesting that tonic excitation is present and is NMDAR mediated. Similar results were obtained with genetic NMDAR inactivation (Zweifel et al. 2009), and lesion of the subthalamic nucleus (STN), a spontaneously active glutamatergic afferent, regularized the firing pattern of dopaminergic neurons.
Fig. 5. Disinhibition bursts may be faster because of depolarization. A: phase plots of spontaneous single-spiking (black) and balanced single-spiking (red). Threshold was defined as the voltage in which there was a significant break (4 × SD) of dV_m/dt from baseline (shown in inset for control) and is shown on the control and balanced phase plots by black and red arrows, respectively. B: there was a significant decrease in maximum dV_m/dt (I) during balanced spiking. There was also a significant increase in minimum dV_m/dt (II) and spiking threshold (III) during balanced spiking. C: overlay of all-points histograms for sampled membrane potentials during spontaneous firing (Control, black bars) and balance between excitation and inhibition (Balanced, white bars). Black and white arrows represent the average membrane potential for Control and Balanced, respectively. The histograms were generated from 9 s of single spike firing in each configuration. Ten superimposed single spikes (aligned at the peak) are shown as an inset for Control (black) and Balanced configurations (red). Scale bars for inset are 10 mV (ordinate) and 100 ms (abscissa). Dashed line in inset shows −40 mV. D: average membrane potential for balanced spiking was more depolarized than control spiking. E: overlay of control (black) and disinhibition (red) bursts (I, same bursts as shown in Fig. 4A). The total current applied by the dynamic clamp is shown above each voltage trace. Summary data show a significant decrease in the time to the peak of the 1st action potential (II) from application of an NMDAR conductance (Control, black) or removal of a GABA_AR conductance (Disinhibition, red). Arrow indicates the time of conductance change. Horizontal lines indicate means for the sample in each panel.
cells (Smith and Grace 1992). Incomplete antagonism of NMDAR activation may explain why the strongly bursting cells of Chergui et al. (1993) do not become silent, as would be expected for a dopaminergic neuron in the high conductance state.

Are the GABA<sub>δ</sub>R and NMDAR conductances applied here with dynamic clamp comparable to the conductances activated by tonic GABAergic synaptic input in vivo? Using a GABA<sub>δ</sub> receptor time constant of 6 ms (τ, Brancucci et al. 2004), a single channel conductance of 5–30 pS (Δg; Guyon et al. 1999; MacDonald and Olsen 1994), and a 50 Hz input rate (F), we calculate that 887–5,330 GABA<sub>δ</sub> receptors must be activated by globus pallidus and substantia nigra pars reticulata inputs to achieve the 8 nS steady-state conductance (g<sub>ss</sub>) used in the balanced configuration in Fig. 1D (g<sub>ss</sub> = Δg/(1 - e<sup>-1/F(τ/F0)</sup>); Wilson et al. 2004). Assuming 12 receptors per synapse (~5 immunogold particles per synapse (Fujiyama et al. 2002) times 2.5 GABA<sub>δ</sub> receptors per gold particle (Nusser et al. 1997)], this corresponds to a minimum ~1–8% of the total GABAergic synapses onto an SNc neuron (~5,600 GABAergic synapses total; Henny et al. 2009). Similarly, we calculate that 2,300 NMDA receptors from 348 synapses must be activated for the total; Henny et al. 2009). Thus the NMDAR/GABA<sub>δ</sub> configuration. Differences in input resistance measurements in vivo (31 MΩ; Grace and Bunney 1983) and in slices (~20 MΩ; 70–250 MΩ, Kita et al. 1986; 135 MΩ, Grace and Omm 1989; 384 MΩ, Paladini et al. 1999b) suggest that 27.3 nS of the total input conductance in vivo (32.3 nS measured in vivo ~ 5 nS measured in slices = 27.3 nS) are caused by tonic NMDA and GABA<sub>δ</sub> conductances. We found that cells went into block at AMPA/GABA<sub>δ</sub> conductances around 4 nS and thus cannot account for the 27.3 nS difference in input conductance. The 27.3 nS input conductance difference in the NMDA/GABA<sub>δ</sub> configuration may be underestimated because of the increase in input resistance with NMDAR activation. The drop in input resistance caused by impalement with sharp electrode recordings will overestimate this difference in input conductance.

An imbalance in tonic inputs may drive the cell into hyperpolarization or depolarization block in some proportion of dopaminergic neurons (Dai and Tepper 1998; Grace et al. 2007). For example, silent cells are cells whose membrane potential (~65 to ~70 mV; Grace and Bunney 1984b) is near the chloride reversal potential (~68 mV; Grace and Bunney 1985). They represent the case where there is a tonic GABA<sub>δ</sub> conductance sufficient to shunt intrinsic pacemaker currents (Grace et al. 2007) but lack a strong enough NMDA conductance to return the cell to the spiking regime. Silent cells, like other shunting configurations, may still be able to produce spikes in response to input fluctuations.

A dopaminergic neuron firing mainly under the influence of a balance between excitatory and inhibitory inputs would be more sensitive to dynamic fluctuations in rate and gain of inputs within the basal ganglia network (Carvalho and Buonomano 2009; Destexhe et al. 2003; Vogels and Abbott 2009) and adopt a more irregular single-spike firing pattern, as normally seen in vivo (Paladini et al. 1999a).

![Figure 6](https://example.com/figure6.png)

**Fig. 6.** The high conductance state of the dopaminergic neuron. The effect of tonic AMPA/GABA<sub>δ</sub> (A) and NMDA/GABA<sub>δ</sub> (B) conductances on the steady-state IV curve of a fictive dopaminergic neuron. Note that the negative slope region below ~65 mV persists at much higher NMDA/GABA<sub>δ</sub> conductances than AMPA/GABA<sub>δ</sub> conductances. The thick black line represents the control condition where g<sub>AMPA</sub> = g<sub>NMDA</sub> and g<sub>GABA</sub> are set to 0. The thin lines represent a 5 nS increase in g<sub>AMPA</sub> (A) or g<sub>NMDA</sub> (B) and a 1.6 nS increase in g<sub>GABA</sub>. Dashed arrows represent displacements of the IV curve as these conductances are increased.
Tipping the scales: generating pauses and bursts

NMDA receptors play an important role in the generation of bursts (Chergui et al. 1993, 1994; Deister et al. 2009; Overton and Clark 1992; Tong et al. 1996; Zweifel et al. 2009). The simplest mechanism by which bursts are generated in vivo is phasic activation of NMDA receptors. However, in the presence of tonic synaptic conductances, this requires that the NMDA receptor currents overcome a substantially reduced input resistance (≤90% from in vitro estimates) and an increased Mg$^{2+}$ block on the NMDA receptor (Paladini and Tepper 1999). These limitations can be overcome by an alternative mechanism where bursting is triggered by disinhibition (Hong and Hikosaka 2008; Jhou et al. 2009; Matsumoto and Hikosaka 2009).

Here we show that not only can bursts be produced by disinhibition but that disinhibition bursts have a greater firing frequency than bursts evoked by NMDA receptors alone. The mechanism by which disinhibition bursts are faster than excitation-only bursts is probably caused by a more depolarized voltage in the balanced state compared with spontaneous, single-spiking (Fig. 5C). This depolarization is a result of the total current produced from the application of NMDA and GABA$_A$ conductances with an effective synaptic reversal potential that is a conductance-weighted average of $E_{\text{NMMA}}$ (0 mV) and $E_{\text{GABA}_A}$ (−60 mV). A depolarized membrane potential may inactivate A-type potassium channels (Gentet and Williams 2007; Khalilq and Bean 2008; Liss et al. 2001). The time to first spike measurement of Fig. 5EI is influenced by both spike-generating sodium channels and A-type potassium channels, which play an important role in the determination of the interspike interval. However, in control conditions, more sodium channels are available, which would overestimate the contribution of $I_N$. This suggests that A-type potassium channels are inactivated in the balanced configuration and may be responsible for the increase in burst frequency. Inactivation of A-type potassium channels would also increase single-spiking frequency (Liss et al. 2001). However, an increase is not seen here (Fig. 3B) because the procedure by which we obtain the balanced configuration was designed to keep the firing rate similar to the control, spontaneous firing rate. These results also suggest that disinhibition bursts may be more precisely timed to rewarding stimuli than bursts by NMDA receptor activation alone.

Recent studies have shown that synaptic plasticity may enhance synaptically triggered burst firing and therefore underlie the acquisition of the burst response to a reward-predicting stimulus (Harnett et al. 2004). Although changes in plasticity can be effected within minutes, changes in the balance between excitatory and inhibitory inputs are capable of modulating bursts quicker. Thus scaling of intraburst firing rate can occur according to the probability of reward associated with a stimulus within a single trial (Morris et al. 2004) or on successive trials of a learning task (Pan et al. 2005).

Phasic increases in GABAergic drive activate postsynaptic GABA$_A$ and/or GABA$_B$ receptors to induce a pause in firing. The length of the pause would be proportional to the increase in drive. GABA$_B$ receptors would be expected to produce a longer pause than GABA$_A$ receptors and may be recruited by synaptic spillover (Galvan et al. 2006). This suggests that the role of GABA$_B$ receptors may not be only presynaptic. Pauses in firing may also be initiated or augmented by removal of excitation (Fig. 4A, e.g., the STN).

The firing pattern of dopaminergic neurons is a behaviorally important signal necessary for normal reward learning (Schultz 1998; Zweifel et al. 2009). Bursts and pauses in firing encode reward prediction error. Our results provide an extrinsic mechanism by which both reward-related responses could be evoked by phasic changes in GABAergic drive. These results suggest that the role of tonic GABAergic inhibition is not simply to suppress single-spiking and burst firing. On the contrary, the presence of tonic background inhibition allows for bidirectional changes in the firing rate and pattern in dopaminergic neurons. Thus GABAergic inhibition plays a fundamental role in the generation of the reward prediction error signal.

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**Disclosures**

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