High Frequency, Short Latency Disinhibition Bursting of Midbrain Dopaminergic Neurons

Abbreviated Title: Disinhibition of Dopaminergic Neurons

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Abstract

During reinforcement and sequence learning, dopaminergic neurons fire bursts of action potentials. Dopaminergic neurons in vivo receive strong background excitatory and inhibitory inputs, suggesting that one mechanism by which bursts may be produced is disinhibition. Unfortunately, these inputs are lost during slice preparation and are not precisely controlled during in vivo experiments. Here we show that dopaminergic neurons can be shifted into a balanced state in which constant synaptic NMDA and GABA_A conductances are mimicked either pharmacologically or using the dynamic clamp. From this state, a disinhibition burst can be evoked by removing the background inhibitory conductance. We demonstrate three functional characteristics of network-based disinhibition that promote high frequency, short latency bursting in dopaminergic neurons. First, we found that increasing the total background NMDA and GABA_A synaptic conductances increased the intraburst firing frequency and reduced its latency. Second, we found that the disinhibition burst is sensitive to the proportion of background inhibitory input that is removed. In particular, we found that high frequency, short latency bursts were enhanced by increasing the degree of disinhibition. Third, the time course over which inhibition is removed had a large effect on the burst, namely that synchronous removal of weak inhibitory inputs produces bursts of high intraburst frequency and shorter latency. Our results suggest that fast, more precisely timed bursts can be evoked by complete and synchronous disinhibition of dopaminergic neurons in a high conductance state.

Introduction

Dopaminergic neurons fire bursts of action potentials during reward (Schultz 2002) and sequence learning (Jin and Costa, 2010). One mechanism by which dopaminergic neurons subjected to background excitatory and inhibitory inputs can generate bursts is disinhibition (Tepper et al., 1995; Paladini et al., 1999b; Lobb et al., 2010). Dopaminergic neurons are bombarded by strong synaptic input from spontaneously active excitatory and inhibitory inputs in vivo (e.g. Grace and Bunney, 1985). The effect of these tonic inputs on the activity of dopaminergic neurons is observed when NMDA or GABA_A receptor antagonists are applied in vivo (Overton and
Clark, 1992; Chergui et al., 1993; Paladini and Tepper, 1999; Brazhnik et al., 2008). Additionally, deafferented dopaminergic neurons recorded in slices do not exhibit the range of firing patterns observed in vivo (Grace and Bunney, 1983; Grace and Bunney 1984a, b; Llinas et al., 1984; Kita et al., 1986; Paladini et al., 1999a,b).

While disinhibition of dopaminergic neurons has been shown in vivo (Gale and Perkel, 2010), a detailed study of disinhibition bursting in intact animals is hindered by the lack of experimental control of the network. We have recently shown that the dynamic clamp (Robinson and Kawai 1993; Sharp et al., 1993) can be used to apply background synaptic conductances to a dopaminergic neuron recorded in slices and that bursts may be evoked with disinhibition (Lobb et al., 2010). In this paper, we utilize the dynamic clamp to quantitatively investigate the functional characteristics of disinhibition bursts evoked by a disinhibitory pathway; for example, the striatum to substantia nigra pars reticulata (SNr) to substantia nigra pars compacta (SNc) pathway, or the striatopallidal/nigral pathway.

Dopaminergic neurons were shifted into a high chord conductance state either pharmacologically with NMDA and GABA_A agonists, or by applying simultaneous NMDA/GABA_A conductance ramps using the dynamic clamp. The high chord conductance state is characterized by an increase in chord conductance without an increase in the slope conductance (see Lobb et al., 2010). In this state, intrinsically generated currents are not shunted and thus are capable of influencing the firing of dopaminergic neurons. Hence, the single spiking activity of dopaminergic neurons in the high chord conductance state remains intact. We then show that high frequency, temporally precise disinhibition bursting produced from dopaminergic neurons in the high chord conductance state depends on several characteristics of disinhibition: the total applied NMDA/GABA_A conductance, the degree of disinhibition, and the degree of synchrony in which the tonic inhibition is removed. Furthered by a quantitative anatomical discussion, our results show that fast, precisely timed bursts can be evoked by disinhibition of dopaminergic neurons in the high chord conductance state.

Materials and Methods
Slice preparation and recordings. Experiments were carried out as previously described (Lobb et al., 2010). All experimental procedures were approved by the University of Texas at San Antonio Institutional Animal Care and Use Committee. Briefly, P17-P28 Sprague Dawley rats (Charles Rivers Laboratories) were anesthetized with isoflurane (3% in O₂), decapitated, and the brains rapidly removed and cooled. 240µm horizontal slices were cut using a vibrating microtome (Microm HM 650V) in oxygenated, cold aCSF and then transferred to an incubation chamber. The cutting aCSF contained (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. The incubation chamber was filled with recording aCSF modified to include 4 mM MgCl₂ and 0.05 mM glutathione. The recording aCSF contained (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 MgCl₂, 2 CaCl₂, 10 dextrose, 25 NaHCO₃, 1.3 ascorbic acid, 2.4 sodium pyruvate.

A slice was then transferred to an intracellular recording rig and superfused with recording aCSF heated to 35°C with an inline heater at a rate of 2ml/min. Presumed SNc neurons were visualized with a gradient contrast imaging system. SNc neurons were patched with a 4-10 MΩ micropipette containing (in mM): 138 K-gluconate, 10 HEPES, 2 MgCl₂, 0.2 EGTA, 0.0001 CaCl₂, 4 Na-ATP, 0.4 Na-GTP. The internal solution was adjusted to a pH of 7.3 using 1M KOH and an osmolarity of 270-275 mOsm. Identified dopaminergic neurons exhibited a slow and pacemaker-like firing pattern (at a frequency of 1-4 Hz), an Iₜ-mediated voltage sag upon passage of hyperpolarizing current, and a prominent spike AHP consistent with previous studies in our lab (Deister et al., 2009; Lobb et al., 2010). Recordings were acquired with a Multiclamp 700B and digitized (Instrutech) under command of the AxographX software program. Dynamic clamp experiments were conducted in whole cell mode as previously described (Deister et al., 2009; Lobb et al., 2010). All recordings were done with a balanced bridge in continuous current clamp (Bridge Mode) using a -6 mV junction potential correction. The equations used to calculate the applied current were:

\[ I_{\text{NMDA}} = -g_{\text{NMDA}} \times \frac{1}{1 + ([\text{Mg}] / 3.57)} \times e^{(V_m \times 0.062)} \times (V_m - E_{\text{NMDA}}) \]

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

where [Mg] = 1.5 mM and E_{NMDA} = 0 mV. For dynamic clamp studies, E_{GABA}_A was set to -60 mV to approximate the physiological reversal potential for GABA_A receptors.
(Gulácsi et al., 2003). For pharmacological studies, $E_{\text{GABA}_A}$ was set to -94 mV as determined by the chloride reversal potential with our solutions (Nernst, 35°C). Synaptic blockers were not used in these experiments as these afferents are removed during slice preparation.

Drugs. Isoguvacine (40μM) and NMDA (40μM) were applied to the slice via superfusion. All drugs were purchased from Tocris.

Data Analysis. Action potentials were detected using a derivative threshold in AxographX (4-5 V/s). Spiking analysis was done using custom code in Mathematica 7 (Wolfram Research Inc.). Maximum intraburst frequency was determined as the reciprocal of the minimum interspike interval (ISI) for all spikes in the analysis window. Mean intraburst frequency was determined as the reciprocal of the mean intraburst ISI in that same window. The time window for analysis was determined by the removal and return of the GABA$_A$ conductance. Frequency analysis for asynchronous disinhibition was performed only on the first second of the two-second disinhibition window. The failure point for spiking in Figure 1 was determined as the total applied conductance of the last spike during the conductance ramp. The failure point is therefore underestimated for the three cells that would have continued spiking with total conductance greater than 80 nS. Convolutions were also done in Mathematica 7. The GABA$_A$ conductance waveform was modeled as:

$$-g*(e^{-t/\tau_{\text{rise}}} - e^{-t/\tau_{\text{decay}}})$$

where $g$ is the single channel conductance of the GABA$_A$ receptor (30 pS), $\tau_{\text{rise}}$ is the rise time constant (0.1 ms), and $\tau_{\text{decay}}$ is the GABA$_A$ deactivation time constant (6 ms). For the current-voltage relationships shown in Figure 1DII, the holding potential was stepped from -60 mV to a command potential (-100 mV to -30 mV at increments of 10 mV) for one second. The average current amplitude in the latter 100 ms of the step was used to calculate the steady state value.

Regression and Statistics. Exponential and Boltzmann functions were used to fit the data in Figure 2C. The following formula was used for exponential fitting: $y = a + b * e^{(g_{\text{total}}/\tau)}$ where $a$ and $b$ are constants, $g_{\text{total}}$ is the total applied conductance ($g_{\text{total}} = g_{\text{NMDA}} + g_{\text{GABA}_A}$) and $\tau$ is the growth/decay time constant. The following formula was used for the Boltzmann function: $y = n_{\text{max}}/(1 + e^{-(g_{\text{total}} - v_{1/2})/k})$ where $n_{\text{max}}$ is the asymptotic
maximum, $v_{1/2}$ is the half maximal conductance, and $k$ is the slope factor. Regression analysis was used to compare the curves fit to maximum and mean burst frequency, latency to first spike, and latency to burst onset data (Lobb et al., 2010). This was implemented in SAS (SAS Institute). Except for mean frequency data, these data were well fit by exponentials and thus a logarithmic transform was applied. For spike counts, a Boltzmann function was used. T-tests were used for statistical comparisons of fit parameters in both groups. Statistical tests to determine whether the slope of a fitted line was non-zero were also performed in Mathematica 7. For all other statistical tests, Prism (Graphpad Software Inc.) was used. All effects are given in terms of mean ± SEM except Figure 2C which is given in terms of mean ± SD. In all experiments presented here, the criterion for statistical significance was set at $p < 0.05$.

**Results**

To consistently provide tonic excitatory and inhibitory inputs to the dopaminergic neuron so that disinhibition could be explored in a reliable way across cells, we applied concurrent NMDA and GABA$_A$ conductance ramps using the dynamic clamp technique to the dopaminergic neuron. The total applied conductance was increased linearly from 0 to 80 nS in 480 seconds. The ratio of NMDA to GABA$_A$ conductance was varied (1:1 to 11:1). An example in which a simultaneous 60 nS NMDA ramp and 20 nS GABA$_A$ ramp are applied is shown in Figure 1A. The average single-spike firing rate during the NMDA/GABA$_A$ ramps was plotted for different ratios (Figure 1B). The total conductance ($g_{\text{NMDA}} + g_{\text{GABA}_A}$) at which single spiking failed during the ramp (its ‘failure point’) is plotted in Figure 1C. Dopaminergic neurons were best able to maintain a constant firing rate at a ratio between 3:1 and 4:1. (Figure 1B,C). These findings agree well with our previous results (Lobb et al., 2010) where the conductance ratio when manually tuned was 3.2:1 across cells. Therefore, a NMDA/GABA$_A$ ratio of 3:1 was chosen for subsequent experiments.

These results were not unique to background synaptic conductances activated from the dynamic clamp. Single spiking persisted after application of 40 μM NMDA and 40 μM isoguvacine (a GABA$_A$ receptor agonist) to a spontaneously firing dopaminergic neuron (Figure 1D). Application of NMDA and isoguvacine depolarized the cell ($p <
0.05, paired t-test, average membrane potential in ACSF: -50.7±1.0; NMDA/Isoguvacine: -45.4±1.8, n=4). Drug application increased the chord conductance of the neuron while having little effect on its slope conductance (Figure 1DII). This differs from the high conductance state described by Destexhe et al. (2003) in which there is an increase in both the chord and slope conductances. Bursts could be evoked by subtraction of a GABA_A conductance with the dynamic clamp (e.g. -2.25 nS in Figure 1E).

We found that single spiking was maintained better with NMDA/GABA_A but not AMPA/GABA_A conductances (data not shown; range of applied ratios was 1:11 to 11:1). Dopaminergic neurons were best able to maintain a constant firing rate for an AMPA/GABA_A conductance ratio of 1:3. The failure points at this 1:3 AMPA/GABA_A conductance ratio were significantly less than the failure points measured with the 3:1 NMDA/GABA_A ratio described above (p < 0.05, unpaired t-test; AMPA/GABA_A: 9.2±1.5 nS; NMDA/GABA_A: 69±7.9 nS; n=5).

Using conductance ramps with a NMDA/GABA_A ratio of 3:1, we investigated how the frequency and timing of disinhibition bursts changed with the total applied conductance (Figure 2). When the total conductance reached 10, 20,…, 80 nS, g_GABA_A was stepped to zero for one second to evoke disinhibition bursts (Figure 2A; n=6). The NMDA conductance continued its slow ramp during this period. In separate experiments, we applied ramps in which no disinhibition bursts were evoked until each of the target total conductances were reached (i.e. 10, 20, …, 80 nS; n=3 for each). Results were similar and the data were pooled (n=9). The maximum and mean frequency of spiking (Figure 2BII) during the disinhibition window increased exponentially with the total applied conductance (maximum: $R^2=0.92$, tau (growth/decay constant) = 41 nS; mean: $R^2=0.90$, tau = 54 nS). The number of spikes that occurred during the disinhibition window also increased with the total applied conductance (Figure 2BII). These data were well fit by a Boltzmann function ($R^2=0.89$; $n_{max}$=12.4 spikes, $v_{1/2}$=15.4 nS, $k$=7.7 nS).

We also observed that the latency to spiking decreased with the total applied conductance. Specifically, the latency to the first spike evoked from disinhibition decreased exponentially with the total applied conductance (Figure 2BIII; $R^2=0.50$, tau = 13 nS). The latency to the first spike occurring in the first ISI less than 80 ms (‘burst
onset”; Grace and Bunney 1984b) also decreased with the total applied conductance ($R^2=0.60$, $\tau = 58 \text{nS}$).

The effects on spiking seen with increases in applied conductance may just be due to an increase in NMDA conductance. To test this, we applied one second, NMDA square conductance pulses (5-70 nS with 5 nS increments) to spontaneously firing dopaminergic neurons ($n=9$). These data were compared with the disinhibition data from Figure 2B in Figure 2C using regression analysis (see Materials and Methods; Lobb et al., 2010). The maximum and mean intra-burst firing rate during disinhibition-induced bursts were significantly faster than the maximum and mean intra-burst firing rate during application of NMDA alone (Figure 2CI; $p < 0.05$, max: 10-60 nS, mean: 10-38 nS; regression analysis). Spike counts (Figure 2CII) could not be transformed so a linear regression could not be performed. However, Boltzmann functions fit to both curves revealed a significant difference in fit parameters between the number of action potentials per burst in the NMDA alone or disinhibition-induced bursts ($p < 0.05$; t-test with pooled variance; $v_{1/2}$: NMDA alone=23.5 nS, disinhibition=11.6 nS; $n_{\text{max}}$: NMDA alone=14.3 nS, disinhibition=12.4 nS; $k$: NMDA alone=10.9 nS, disinhibition=5.80 nS). Both the latency to the first spike (Figures 2CIII) and the latency to burst onset (data not shown) of disinhibition bursts were significantly shorter in comparison to NMDA alone bursts ($p < 0.05$, 10-60 nS; regression analysis). These results suggest that increasing the total background NMDA/GABA$_A$ conductances promotes high frequency, short latency bursts.

In the preceding experiments, the GABA$_A$ conductance was set to zero during the disinhibition period. However, in vivo disinhibition may not be complete, but rather reduced to some non-zero level provided by the remaining inhibitory influences. Therefore, we investigated whether the degree of disinhibition achieved during the disinhibition period had an effect on disinhibition burst frequency. 3:1 NMDA/GABA$_A$ 240 s conductance ramps with a total maximal conductance of 40 nS (30 nS NMDA, 10 nS GABA$_A$) were applied to put the dopaminergic neuron in the high chord conductance state. At 30 s intervals, $g_{\text{GABA}}$ was stepped for one second to a new value that ranged from 10% to 100% of the original $g_{\text{GABA}}$ (i.e. 1 to 10 nS). $g_{\text{NMDA}}$ remained constant during those steps. This was done in an ascending ($n=6$) or a random order ($n=3$). Data
were similar and therefore pooled (n=9). An example is shown in Figure 3A. Lines fit (not shown) to burst frequencies (p < 0.05; slope of maximum burst frequency = 24 s⁻¹; slope of mean burst frequency = 13 s⁻¹) and the number of spikes (p < 0.05; slope = 11) had nonzero slopes and increased linearly with the degree of steady state disinhibition (Figure 3BI-II; n=9). The latency of burst onset did not change significantly with increased disinhibition (Figure 3BIV; p>0.05) due the large variability encountered (linear fit: R²=0.0092). However, the latency to the first spike upon disinhibition decreased and became less variable with the degree of steady state disinhibition (Figure 3BIII; p<0.05; n=9).

We next investigated the time course for the removal of a tonic inhibitory input on the frequency and temporal precision of disinhibition bursts in dopaminergic neurons. We assume a tonic NMDA-mediated input (e.g. subthalamic nucleus, STN; Chergui et al., 2004) and a tonic GABA_A-mediated input (e.g. SNr, Paladini et al., 1999a; see Materials and Methods). Periodic, weak synaptic input will summate in time with an average steady-state conductance given by the equation (e.g. Wilson et al., 2004):

\[ g_{ss} = \frac{\Delta g}{(1-e^{-1/(\tau F_n)})} \]

where \( \Delta g \) is the conductance increment per activated receptor (GABA_A: 30pS, Macdonald and Olsen 1994; Guyon et al., 1999; NMDA: 50pS, Edmonds et al., 1995), \( \tau \) is the deactivation time constant (GABA_A: 6mS, Brancucci et al., 2004; NMDA: 43 ms, Schilström et al., 2006), \( F \) is the firing frequency of the presynaptic neuron (rat SNr: 30 Hz, Deniau et al., 1978, Celada et al., 1999; rat STN: 5 Hz, Chergui et al., 2004), and \( n \) is the number of postsynaptic receptors activated from activity in afferent nuclei. Thus the 30 nS NMDA conductance and 10 nS GABA_A conductance used above corresponds to the activation of 2788 NMDA receptors and 1849 GABA_A receptors. The overall time course for disinhibition differs depending on whether disinhibition is synchronous (Figure 4A) or asynchronous (Figure 4B). Complete and synchronous removal of inhibition causes the tonic inhibitory drive to decay according to the deactivation time constant for each GABA_A receptor. If the deactivation time constant for each of the 1849 GABA_A receptors are the same then the tonic inhibitory drive decays exponentially according to that deactivation time constant. This is illustrated in Figure 4A as the convolution of a step function and a GABA_A conductance waveform with a 6 ms
deactivation time constant. However, if removal of inhibition is asynchronous and occurs
over a time course longer than the GABAₐ deactivation time constant then the time
course of disinhibition follows the time course in which inhibition is removed (Figure
4B). If the disinhibition activating process (i.e. the inhibitory nuclei which when
activated causes disinhibition through a tonically active intermediary) is normally
distributed then the time course of disinhibition is well fit by the complementary
cumulative distribution function. This function is described by the mean (μ) and standard
deviation (σ) of the activating normal distribution:

\[ 1 - \left(1 + \frac{t - \mu}{\sigma \sqrt{2}}\right)/2, \]

where Erf is the error function and t is time.

Disinhibition caused by synchronous removal of GABAₐ receptor activation will
occur with a time course that is approximated by the GABAₐ deactivation time constant
(Figure 4A). The deactivation time constant of miniature IPSCs recorded in dopaminergic
neurons is approximately 6 ms (Brancucci et al., 2004). Desensitization may increase the
deactivation time constant (Jones and Westbrook 1995); however, it is unclear how much
desensitization of GABAₐ receptors will occur on dopaminergic neurons in vivo in
response to a tonic inhibitory input. To explore this, gₐ was removed with a simple
exponential decay rather than stepwise (Figure 5). NMDA/GABAₐ conductance ramps
(3:1 ratio, 40 nS total) were applied to dopaminergic neurons (as described in Figure 1).
At regular 30 sec intervals, a one second disinhibition pulse was applied such that gₐ decayed to 0 with a given time constant. The range of disinhibition time constants applied
ranged from 1 to 100ms and were given in a random order. A representative example is
shown in Figure 5A. Maximum burst frequency decreased as disinhibition time constant
increased (Figure 5B; p < 0.05, slope = -30 s⁻², n=8). The mean frequency of the burst
(Figure 5B; p > 0.05, slope = -8 s⁻², n=8) and the number of spikes in the burst (Figure
5C; p > 0.05, slope = -12 s⁻¹, n=8) were unchanged. There was no difference between
bursts evoked by stepwise disinhibition described previously and bursts evoked with a ~6
ms disinhibition time constant (p > 0.05, unpaired t-test; maximum burst frequency for
5.4 ms τₜ, 16±1.6 (n=8), stepwise 22±3 (n=18; data pooled from Figure 2, 40 nS and
Figure 3 100% disinhibition); mean burst frequency for 5.4 ms τₜ, 13±1.5 (n=8), stepwise
14±1.3 (n=18)). The latency to the first spike increased with the disinhibition time
constant (Figure 4D; \( p < 0.05 \), slope=0.34, \( n=8 \)). The latency to the burst also increased with the disinhibition time constant (\( p < 0.05 \), slope=1.6, \( n=8 \)).

Disinhibition caused by asynchronous removal of GABA_A receptor activation will occur with a time course that is approximated by the complementary cumulative distribution function, which is described by its mean and standard deviation (Figure 4A). The mean will determine when the burst occurs, and thus a change in mean will shift the onset of the burst in time. The standard deviation will have a large effect on the shape and time course of the conductance decay. We then investigated what the effect the standard deviation of the complementary cumulative distribution function would have on the bursts produced by disinhibition (Figure 6).

NMDA/GABA_A conductance ramps totaling 40 nS were applied to dopaminergic neurons as before. At thirty-second intervals, \( g_{GABA_A} \) was removed according to the complementary cumulative distribution function. The mean \( \mu \) was fixed but the standard deviation \( \sigma \) was varied between 1 and 300 ms in a random order. A representative example is shown in Figure 6A-B.

Varying \( \sigma \) had a significant effect on the frequency of the disinhibition burst. Maximum and mean burst frequency decreased linearly with \( \sigma \) (Figure 6C; maximum burst frequency slope = -28 s^{-2}, \( p < 0.05 \); mean burst frequency, slope = -15 s^{-2}, \( p < 0.05 \); \( n=6 \)). The slopes of lines fit to maximum and mean burst frequency were significantly different from one another (\( p < 0.05 \), t-test). The number of spikes in the disinhibition burst also decreased with increasing \( \sigma \) (Figure 6D; \( p > 0.05 \); slope=14 s^{-1}, \( n=6 \)).

Varying \( \sigma \) also affected the shape of the disinhibition burst. The interspike intervals of disinhibition bursts with a small \( \sigma \) (e.g. 1 ms) progressively increased during the burst (Figure 6A, top; 6B,E). The interspike intervals of disinhibition bursts with large \( \sigma \) (e.g. 300 ms) progressively decreased until the middle of the burst, where the interspike intervals began to progressively increase (Figure 6A, bottom; 6B,E). The difference in ISI between \( \sigma =1 \) and \( \sigma =300 \) ms persisted out to the third ISI in the sequence (Figure 6F). Both the latency to first spike (\( p < 0.05 \), slope=0.85, \( n=6 \)) and the latency to burst onset increased with \( \sigma \) (Figure 6D; \( p < 0.05 \), slope=1.82, \( n=6 \)).

To determine whether dopaminergic neurons responded differently to an exponential decay (\( \tau_d \); Figure 5) or to a decay governed by the standard deviation of the
complementary cumulative distribution function ($\sigma$; Figure 6), the regression slopes calculated above for maximum and mean burst frequency, number of spikes elicited, latency to first spike and latency to burst onset were compared. Only the latency to first spike was statistically significant ($p < 0.05$; unpaired t-tests with pooled variance). Regression analysis confirmed that the latency to first spike for disinhibition described by the complementary cumulative distribution function was significantly greater than disinhibition occurring with an exponential decay ($p < 0.05$; $\tau_d/\sigma \geq 70$ ms).

**Discussion**

Dopaminergic neurons fire bursts of action potentials in response to salient stimuli (Schultz et al., 1997; Horvitz et al., 1997; Redgrave et al., 1999), when a greater than expected reward is received (Schultz et al., 1997), or during sequence learning (Jin and Costa, 2010). Burst firing in midbrain dopaminergic neurons may be caused by phasic activation of AMPA or NMDA receptors (Deister et al., 2009; Zweifel et al., 2009; Blythe et al., 2009; Lobb et al., 2010). However, neither AMPA nor NMDA receptor activation may be well suited to produce bursts with high temporal precision due to the presence of a strong tonic GABAergic input in vivo (Grace and Bunney 1985; Paladini and Tepper 1999; Brazhnik et al., 2008) and A-type potassium channels (Liss et al., 2001). Additionally, NMDA receptors activate slowly, with a rise time of 5-10 ms (e.g., Dalby and Mody 2003).

Bursts produced by disinhibition produce high frequency bursting at short latencies (Lobb et al., 2010). Disinhibition bursts also require NMDA receptor activation; however, NMDA receptors are employed as a source of tonic excitation. It is possible that separate populations of dopaminergic neurons can evoke different types of bursts. Single dopaminergic neurons in the high chord conductance state may also be able to employ both phasic AMPA or NMDA receptor activation, and disinhibition to drive bursting. The capacity of a neuron to fire both phasic NMDA bursts and disinhibition bursts will depend largely on the number of NMDA receptors that are not activated by tonic inputs. If the majority of NMDA receptors are activated tonically then it is less likely that the remaining inputs will generate the large NMDA conductance required to generate a burst. In these cases, it is more probable that a single dopaminergic neuron in the high conductance state will fire bursts only by disinhibition. On the other hand, if
there are sufficient numbers of NMDA receptors that are not activated by tonic inputs and that can overcome strong tonic inhibition (e.g. Grace and Bunney, 1985), then it is possible that the neuron can generate both phasic NMDA bursts and disinhibition bursts.

Our results demonstrate three aspects of disinhibition that promote high frequency bursts of short latency: 1) increased background conductances, 2) completeness of disinhibition, and 3) synchronous removal of tonic inhibition.

**Increased Background Conductances**

The activity of dopaminergic neurons in vivo depends on the dopaminergic neuron’s intrinsic pacemaking currents acting in tandem with tonic NMDA and GABA<sub>A</sub>-mediated synaptic currents. Our results suggest that a $g_{\text{NMDA}}$ to $g_{\text{GABA}}$ close to 3:1 will yield a state in which single spiking can be maintained with a minimal change in firing rate. We do not yet know the level of tonic inputs *in vivo*, or the contributions of $g_{\text{NMDA}}$ to $g_{\text{GABA}}$ to the total background synaptic conductance imposed by these inputs. At a 3:1 ratio, the effective reversal potential is depolarizing (see also Lobb et al., 2010). As $g_{\text{total}}$ is increased during the ramp and the cell depolarizes, the increase in input resistance seen with NMDAR activation (Koch, 1999) is lost and the intrinsic conductances are shunted. This explains why single spiking frequency falls off with increased $g_{\text{total}}$ (Figure 1B).

We found an increase in intraburst frequency and decrease in latency with increasing $g_{\text{total}}$. This can be partially accounted for by the increased NMDA receptor conductance at the moment of disinhibition (Figure 2C). The remainder is probably due to increased inactivation of A-type potassium currents with depolarization (see Lobb et al., 2010). The pause in firing after the offset of disinhibition also increases with the total background synaptic conductance. This may due to the activation of a sodium pump as a result of sodium accumulation (Johnson et al., 1992).

**Completeness of Disinhibition**

Tonic GABAergic input onto SNc dopaminergic neurons arises predominately from afferents such as the globus pallidus (GP), endopeducular nucleus (EP), SNr, and rostral tegmental nucleus (reviewed in Tepper and Lee, 2007; Jhou et al., 2009). The relative contribution of inhibition provided from each afferent structure is unknown. Our
results show that the intra-burst frequency and latency of bursting depends on the degree of disinhibition achieved.

Striatal projection neurons are often described as either direct or indirect pathway neurons projecting to the SNr and GP respectively (e.g. Albin et al. 1989). However, axons of single striatal neurons both in the rat and monkey send collaterals to multiple axonal targets (Kawaguchi et al., 1990; Parent et al., 1995). Our results suggest that an important role of collateralization may be during disinhibition. For example, type IIa projection neurons in the rat project to the GP, EP and SNr (Kawaguchi et al., 1990). Phasic activation of type IIa cells would remove three of the four sources of tonic inhibition described above and would thus be expected to produce greater disinhibition bursts in dopaminergic neurons than type I neurons, which only innervate the GP.

Synchronous versus Asynchronous Disinhibition

The frequency and latency of the burst depends on the time course of disinhibition. Synchronous removal causes the tonic GABA<sub>A</sub>-mediated input to decay according to the deactivation time constant of the GABA<sub>A</sub> receptor. Asynchronous removal of tonic GABA<sub>A</sub>-mediated inputs by a Poisson process evolves according to the complementary cumulative distribution function (Figure 4). The burst will be shifted in time according to the mean of the Gaussian distribution. The shape, frequency and latency of the disinhibition burst are strongly affected by its standard deviation. The mean and the standard deviation are determined by the asynchronous generation of action potentials of medium spiny neurons (Stern et al., 1998) and differences in axonal transmission delays.

Implications for Reward Learning

Many models of reinforcement learning encode reward-related learning through plastic changes at corticostriatal synapses (Houk et al., 1995, Wickens and Kotter 1995). The efficacy of these synapses is dependent on the relative timing of cortical to striatal spiking and phasic increases in dopamine. According to this hypothesis, synapses that contribute to the selection of the rewarded response undergo LTP while synapses that did not contribute to selection undergo LTD. Thus the corticostriatal synapses encoding the
stimulus or expect reward outcome during stimulus-response and action-outcome learning are strengthened (Horvitz et al., 2009). Previous results have shown that LTP occurs upon concurrent activation of presynaptic corticostriatal terminals and postsynaptic medium spiny neurons when paired with a phasic increase in dopamine (Wickens et al., 1996). If activation of medium spiny neurons generates a disinhibition burst in SNc dopaminergic neurons then our results will show that a high frequency burst will occur at short latencies. This is expected to produce greater changes in synaptic efficacy than if the dopamine signal were delayed.

The striatum may have a causal role in the generation of reward signaling of dopaminergic neurons. Phasic activation of both medium spiny neurons of the striatum and dopaminergic neurons increases according to reward prediction error (Oyama et al., 2010). One hypothesis is that the direct projection from the striatum to the SNc (Bolam and Smith 1990) may be important for the generation of an inhibitory reward prediction signal (Houk et al., 1995). However, this hypothesis predicts an inhibitory period during the delay between cue and reward. Fiorillo et al. (2003) has shown that there is a sustained increase in firing during the delay period. Furthermore, none of the medium spiny neurons sampled by Oyama et al. (2010) were phasically activated on trials in which reward was not received. Another hypothesis is that activation of striatum evokes disinhibition bursts in SNc cells. The striatonigral pathway is necessary for reward learning (Hikida et al., 2010). Striatal cells are phasically activated at the time of salient stimulus (e.g. Sedgwick and Williams 1967; Hikosaka et al., 1989a; Schulz et al., 2009), a reward-predicting cue (Cromwell and Schultz 2003; Oyama et al., 2010) or at the time of reward (Hikosaka et al., 1989b; Oyama et al., 2010). Activation of these cells would inhibit neurons in the GP, EP and SNr (e.g. Kimura et al., 1984; Shin and Sommer, 2010). Similar results have been found in songbirds (Gale and Perkel, 2010). Concurrent activation of the STN (Darbaky et al., 2005; Lardeux et al., 2009) or superior colliculus (Comoli et al., 2003) would further increase the frequency of the disinhibition burst, or reduce its latency.

Disinhibition is an effective mechanism for generating bursts in dopaminergic neurons. The temporal precision of the burst as well as its intraburst frequency is
determined by specific characteristics of the disinhibitory process. We have demonstrated that high frequency, precisely timed bursts can be generated by a complete and synchronous disinhibition of dopaminergic neurons in the high chord conductance state. As a result, disinhibition bursts could act as a robust and scaleable teaching signal to the striatum during reward and sequence learning.

References


Paladini CA, Tepper JM (1999). GABA(A) and GABA(B) antagonists differentially affect the firing pattern of substantia nigra dopaminergic neurons in vivo. Synapse. 32:165-76.


Figure Legends:

**Figure 1:** Dopaminergic neurons in a high chord conductance state with NMDA/GABAA receptor activation. A. Concurrent NMDA and a GABAA conductance ramps were applied to a dopaminergic neuron in a whole-cell recording. A fixed 3:1 NMDA to GABAA ratio is maintained during the ramp. The ramp begins at t=5 s and is completed at t=485 s with a total conductance (g_{NMDA}+g_{GABAA}) of 80nS. Spontaneous activity resumes after both conductances are removed. B. Mean single spiking frequency during application of conductance ramps for a series of fixed NMDA to GABAA conductance ratios (number of cells shown in C). C. The conductance at which single spiking fails during the conductance ramp (“failure point”) is plotted for the different NMDA:GABAA conductance ratios. The numbers of cells for each ratio are shown in parenthesis. D. Single spiking persists after application of 40 μM NMDA and 40 μM isoguvacine (a GABA_A receptor agonist) (I). The steady state IV curve of the dopaminergic neuron is shown in panel DII for control ACSF (black), after application of NMDA and isoguvacine (red), and post-drug ACSF (blue). The shape of the IV curve largely remains unchanged with drug application. E. The dopaminergic neuron in the pharmacologically-induced high chord conductance state can generate bursts of action potentials in response to subtraction of a GABA_A receptor conductance with the dynamic clamp (19.4 Hz maximum burst frequency; 13.4 Hz mean burst frequency). Panels D and E are from the same cell.

**Figure 2:** Disinhibition burst frequency increases as the total applied NMDA/GABAA conductance increases. A. Concurrent NMDA and GABAA conductance ramps (3:1 ratio) are applied to a dopaminergic neuron in a representative example. As the total applied conductance reaches 10, 20, ..., 80 nS, the GABAA conductance is phasically set to zero for one second. Insets show the change in firing at 20, 40, 60, and 80 nS total conductance. B shows summary data for the maximum and mean frequency (I), number of spikes (II), and latency to the first spike (III), during the one second disinhibition window as a function of the total applied conductance (n=9). Burst onset was defined as the latency of the first spike in the first ISI of less than 80 ms (cells in which this criteria were not met were excluded from the mean calculation for that conductance). C. The maximum firing frequency (I), number of spikes (II), and latency to first spike (III) for disinhibition bursts (black) are shown along with bursts evoked from phasic NMDA receptor activation alone (gray). The NMDA conductance of the disinhibition burst was 0.75 times the total applied conductance. NMDA only bursts were evoked by a one second, NMDA conductance step (5-70nS, 5nS increment) in a spontaneously firing dopaminergic neuron.

**Figure 3:** Burst frequency increases as the degree of disinhibition increases. A. Concurrent NMDA and GABAA conductance ramps (3:1 ratio; not shown) are applied to a dopaminergic neuron in a representative example. A total of 40nS (g_{NMDA}=30nS, grey; g_{GABAA}=10nS, black) is reached at the end of the ramp and is held steady. In one second windows, a proportion of g_{GABAA} is removed causing a phasic increase in firing. B shows summary data for the (I) maximum (black) and mean (grey) frequency of spikes, (II)
number of spikes, and (III) latency to first spike as a function of the proportion of \( g_{\text{GABA}_A} \) removed (n=9).

**Figure 4: Modeling the Time Course of Disinhibition of SNc Dopaminergic Neurons.**

A source of tonically active inhibitory inputs is turned off synchronously (A) or asynchronously (B). This is represented by a step function (A, left) or the complementary cumulative distribution function (B; \( f(t) = cCDF (\mu = 1.0 \text{ s}, \sigma = 100 \text{ ms}) \), left). Each input is described by a conductance waveform (\( g(t) \), middle) with a deactivation time constant of 6 ms. The time course of disinhibition is determined by their convolution \([f *g](t)\). Thus tonic inhibition decays according to the GABA\(_A\) deactivation time constant (A, synchronous) or the complementary cumulative distribution function (B, asynchronous). The time at which an individual GABA\(_A\) input in the asynchronous case is removed is taken from a normal distribution.

**Figure 5: Burst frequency decreases as the time constant of disinhibition increases.**

A. Concurrent NMDA and GABA\(_A\) conductance ramps (3:1 ratio) were applied to a dopaminergic neuron in a representative example (as in Figure 1). A total conductance of 40nS (\( g_{\text{NMDA}}=30\text{nS, grey}; g_{\text{GABA}_A}=10\text{nS, black} \) was achieved at the end of the ramp and maintained. In one second intervals, \( g_{\text{GABA}_A} \) was removed according to a simple exponential decay, \( 10*e(-t/\tau_d) \). The disinhibition time constant (\( \tau_d \) was varied. B. Unlike mean burst frequency (\( p>0.05, n=8 \)), the maximum burst frequency showed a significantly non-zero slope across the range of \( \tau_d \) values (\( p < 0.05, n=8 \)). C. The total number of spikes in the disinhibition window was not changed with different \( \tau_d \) values (\( p < 0.05, n=8 \)). D. The latency to the initiation of the burst, defined as an ISI less than 80ms, increased significantly with \( \tau_d \) values (\( p < 0.05, n=8 \)).

**Figure 6: Disinhibition bursts change in frequency and shape with asynchronous removal of the GABA\(_A\) conductance.** A. Concurrent NMDA and GABA\(_A\) conductance ramps (3:1 ratio) were applied to a dopaminergic neuron in a representative example (as in Fig 1). A total conductance of 40nS (\( g_{\text{NMDA}}=30\text{nS, grey}; g_{\text{GABA}_A}=10\text{nS, black} \) was achieved at the end of the ramp and maintained. At thirty-second intervals, \( g_{\text{GABA}_A} \) was removed according to the complementary cumulative distribution function, which describes the asynchronous removal of a population of tonically activated GABA\(_A\) receptors. The standard deviation parameter of that function is described by \( \sigma \). A raster plot of disinhibition spiking as sampled in A is shown in B. The maximum (black) and mean (grey) frequency of the burst (C) as well as the number of spikes in the burst (D) decreased significantly with increasing \( \sigma \) (\( p < 0.05, n=6 \)). E-G. The shape of the disinhibition bursts changed with increasing \( \sigma \). Changes in the interspike interval as the burst progresses in all six cells. With small \( \sigma \) (i.e. more synchronous \( g_{\text{GABA}_A} \) removal), disinhibition bursts showed a progressive increase in the ISI of each spike in the burst (B, E black). With large \( \sigma \) (more asynchronous \( g_{\text{GABA}_A} \) removal), disinhibition bursts showed a progressive decrease in the ISI of each spike in the burst (B, E gray). The first ISI less than 80 ms (dashed line in C) defines the onset of the burst. F. There was a significant increase in the interspike interval up to ISI number 3 for \( \sigma = 1 \text{ ms} \) when compared to \( \sigma =300 \text{ ms} \) (\( p < 0.05, n=6 \)). G. The latency at which the burst began increased with \( \sigma \) (\( p < 0.05, n=6 \)).
A 3:1 NMDA:GABA<sub>A</sub>

B

C

D

E

NMDA + Isoguvacine

Return to ACSF

ACSF

\[ g_{\text{NMDA}} = 60 \text{ nS} \]

\[ g_{\text{GABA}} = 20 \text{ nS} \]

Failure Point (nS; mean±SD)

Ratio

\[ 0.167 \text{ nS/sec} \]

\[ g_{\text{NMDA}} \]

\[ g_{\text{GABA}} \]

Vm (mV)

Current (pA)

\[ g_{\text{GABA}} = -2.25 \text{ nS} \]
A

Synchronous Disinhibition

Convolution

B

Asynchronous Disinhibition

Convolution