Synaptic Activation of Dendritic AMPA and NMDA Receptors Generates Transient High-Frequency Firing in Substantia Nigra Dopamine Neurons

In Vitro

Sarah N. Blythe, Jeremy F. Atherton, and Mark D. Bevan

Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

Submitted 31 October 2006; accepted in final form 14 January 2007

Blythe SN, Atherton JF, Bevan MD. Synaptic activation of dendritic AMPA and NMDA receptors generates transient high-frequency firing in substantia nigra dopamine neurons in vitro. J Neurophysiol 97: 2837–2850, 2007. First published January 24, 2007; doi:10.1152/jn.01157.2006. Transient high-frequency activity of substantia nigra dopamine neurons is critical for striatal synaptic plasticity and associative learning. However, the mechanisms underlying this mode of activity are poorly understood because, in contrast to other rapidly firing neurons, high-frequency activity is not evoked by somatic current injection. Previous studies have suggested that activation of dendritic N-methyl-D-aspartate (NMDA) receptors and/or G-protein-coupled receptor (GPCR)-mediated reduction of action potential afterhyperpolarization and/or activation of cation channels underlie high-frequency activity. To address their relative contribution, transient high-frequency activity was evoked using local electrical stimulation (1 s, 10–100 Hz) in brain slices prepared from p15–p25 rats in the presence of GABA and D2 dopamine receptor antagonists. The frequency, pattern, and morphology of action potentials evoked under these conditions were similar to those observed in vivo. Evoked activity and reductions in action potential afterhyperpolarization were diminished greatly by application of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or NMDA receptor selective antagonists and abolished completely by co-application of AMPA and NMDA antagonists. In contrast, application of glutamate and cholinergic GPCR antagonists moderately enhanced evoked activity. Dendritic pressure-pulse application of glutamate evoked high-frequency activity that was similarly sensitive to antagonism of AMPA or NMDA receptors. Taken together, these data suggest that dendritic AMPA and NMDA receptor-mediated synaptic conductances are sufficient to generate transient high-frequency activity in substantia nigra dopamine neurons by rapidly but transiently overwhelming the conductances underlying action potential afterhyperpolarization and/or engaging postsynaptic voltage-dependent ion channels in a manner that overcomes the limiting effects of afterhyperpolarization.

INTRODUCTION

In vivo, substantia nigra dopamine neurons exhibit repetitive, low-frequency (<5 Hz), regular/irregular activity (Celada et al. 1999; Grace and Bunney 1983a,b; Hyland et al. 2002; Paladini and Tepper 1999; Schultz 2002; Wilson et al. 1977). Unexpected items of biological value (“rewards”), such as food, can, however, rapidly elicit (within 100 ms) transient (<200 ms duration), high-frequency (>15–20 Hz) firing (Hyland et al. 2002; Schultz 2002). Consistent pairing of sensory information with rewards can also lead to the generation of high-frequency activity that encodes the reward predictability of sensory information (Morris et al. 2004; Schultz 2002). Transient high-frequency activity of substantia nigra dopamine neurons may therefore be important for associative learning (Dayan and Balleine 2002; Schultz 2002). Indeed this pattern of activity leads to a profound increase of dopamine in the striatum (Chergui et al. 1994b; Cragg et al. 2000; Wightman and Robinson 2002), which in turn facilitates the long-term modification of synaptic transmission (Calabresi et al. 2000; Gerdeman et al. 2002; Kreitzer and Malenka 2005; Reynolds et al. 2001; Wang et al. 2006).

The cellular mechanisms underlying transient high-frequency firing or “burst firing” of dopamine neurons (as it is commonly referred to) have been difficult to understand because, in contrast to other types of cell that are capable of high-frequency firing (e.g., Bevan and Wilson 1999; Kawaguchi 1993), somatic current injection in vitro/in vivo rarely elevates firing frequency >10 Hz due to depolarization block (Grace and Bunney 1983a,b, 1984b; Kita et al. 1986; Richards et al. 1997). Because substantia nigra dopamine neurons exhibit only low-frequency activity in de-afferented slice preparations (e.g., Harris et al. 1989; Kita et al. 1986; Lacey et al. 1987; Richards et al. 1997; Sanghera et al. 1984; Shepard and Bunney 1991; Wolfart et al. 2001; Yung et al. 1991), synaptic inputs presumably drive high-frequency activity. Indeed, stimulation of glutamatergic afferents arising from either the thalamic nucleus (Chergui et al. 1994a), the prefrontal cortex (Tong et al. 1996), or the pedunculopontine nucleus (Lokwan et al. 1999) elicits transient high-frequency activity in vivo, which in some cases is partly attenuated by N-methyl-D-aspartate (NMDA) but not alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists (Chergui et al. 1994a; Tong et al. 1996). Spontaneous transient high-frequency activity in anesthetized animals (Chergui et al. 1993, 1994a; Grace and Bunney 1983a; Overton and Clark 1992; Paladini and Tepper 1999; Smith and Grace 1992) also appears to be partly sensitive to blockade of NMDA receptors (Chergui et al. 1993; Overton and Clark 1992). Although locally applied AMPA receptor antagonists have minimal effects on the high-frequency activity of dopamine neurons in vivo, brief application of AMPA receptor agonists reliably generates high-frequency firing both in vivo (Christoffersen and Melzer 1995; Zhang et al. 1994) and in vitro (Kim et al. 2004), and firing responses to brief electrical stimulation of afferents such as the pedunculopontine nucleus are antagonized by AMPA receptor but not NMDA antagonists (Di Loreto et al. 1992). Bath application of NMDA and injection of tonic hyperpolarizing current can elicit high-frequency activity in a subset (<30%) of dopamine neurons in vitro (Cannavaro 1999; Johnson et al. 1992; Li et al. 1996), but this activity is distinct from the
pattern observed in vivo because the frequency of action potentials increases rather than decreases during each bout of high-frequency firing. High-frequency activity of substantia nigra dopamine neurons has also been reported in response to ionophoresis of aspartate onto dendrites or local high-frequency (8 stimuli at 60 Hz) stimulation using cell-attached recordings. However, the similarity of this activity pattern to that observed in vivo has not been thoroughly addressed, and although the evoked activity was in part dependent on NMDA receptor activation, the contribution of AMPA receptors was not tested (Morikawa et al. 2003).

A recent theoretical study supports a critical role for dendritic NMDA receptor activation in transient high-frequency firing (Kuznetsov et al. 2006). The rhythmic low-frequency activity of substantia nigra dopamine neurons (in the absence of synaptic input) is driven by an intrinsic oscillation (Fujimura and Matsuda 1989; Grace and Bunney 1984a; Harris et al. 1989; Kang and Kitai 1993a,b; Kita et al. 1986; Mercuri et al. 1994; Nedergaard et al. 1993; Neuhoff et al. 2002; Ping and Shepard 1996; Shepard and Bunney 1991; Wilson and Callaway 2000; Wolfart et al. 2001): dihydropyridine-sensitive voltage-dependent Ca\(^{2+}\) (Cav) channels that drive the depolarizing phase of the cycle lead to an elevation of intracellular Ca\(^{2+}\) which in turn activates small-conductance Ca\(^{2+}\)-dependent K\(^+\) (SK) channels that underlie the hyperpolarizing phase of the oscillation and in part dictate its frequency. Although Cav and SK channels (and AMPA and NMDA receptors) (Paquet et al. 1997) are expressed throughout the entire somatodendritic axis, the frequency of oscillation is inversely related to the diameter of each compartment due to the clearance rate of intracellular Ca\(^{2+}\), which is related to surface area:volume ratio (Kuznetsov et al. 2006; Wilson and Callaway 2000). In the absence of synaptic input, the neuron’s frequency of oscillation is restricted by the largest compartment, the soma, due to the relative magnitude of the hyperpolarizing phase of its oscillation (Kuznetsov et al. 2006; Wilson and Callaway 2000). However, transient recruitment of dendritic voltage-dependent NMDA receptors by glutamatergic synaptic inputs (Paquet et al. 1997) has been proposed to boost the influence of dendrites by amplifying their oscillation and thus driving high-frequency firing (Kuznetsov et al. 2006). In contrast, dendritic AMPA receptor activation (in isolation) is thought to be incapable of generating high-frequency activity due to depolarization block of dendrites (Kuznetsov et al. 2006).

Other mechanisms have been hypothesized to contribute to the generation of transient high-frequency activity including G-protein-coupled receptor (GPCR)-mediated reductions in afterhyperpolarization and/or the activation of cation-permeable channels (Bengston et al. 2004; Johnson and Wu 2004; Kita et al. 1999; Lacey et al. 1990; Overton and Clark 1997; Prisco et al. 2002; Scroggs et al. 2001; Shen and Johnson 1997). Indeed blockade of SK channels or bath application of GPCR agonists greatly increase the incidence and intensity of high-frequency firing during application of NMDA (Johnson and Wu 2004; Prisco et al. 2002). However, activation of glutamatergic, cholinergic, and norepinephrergic GPCRs through synaptic stimulation or brief application of exogenous agonists generates a slow inhibitory postsynaptic potential (IPSP), due to the release of Ca\(^{2+}\) from intracellular stores and the subsequent activation of SK channels (Fiorillo and Williams 1998, 2000; Morikawa et al. 2003; Paladini and Williams 2004). Only prolonged activation of GPCRs, which leads to the exhaustion of intracellular Ca\(^{2+}\) stores, appears to favor the activation of cation channels (Bengston et al. 2004; Lacey et al. 1990; Prisco et al. 2002; Shen and Johnson 1997).

The primary objective of this study was therefore to resolve the relative contributions of excitatory synaptic inputs acting at AMPA receptors, NMDA receptors, and GPCRs to transient high-frequency firing. Using an in vitro brain slice preparation, we found that local stimulation of afferents could evoke transient high-frequency firing that was similar in action potential properties to “burst firing” reported in vivo (Chergui et al. 1993, 1994a; Grace and Bunney 1983a; Overton and Clark 1992; Paladini and Tepper 1999; Smith and Grace 1992). The majority of our recordings were carried out using the gramicidin-based perforated patch-clamp configuration to minimize the perturbation of postsynaptic voltage-dependent channels, intracellular Ca\(^{2+}\) dynamics, and GPCR signaling (Brown et al. 1989; Neher and Augustine 1992; Suh et al. 2004; Velumian and Carlen 1999; Velumian et al. 1997; Zhang et al. 1994, 1995). In vitro conditions also permitted us to continuously block inhibitory synaptic transmission at GABA (Gulacsi et al. 2003; Haussser and Yung 1994; Iribe et al. 1999) and dopamine (Beckstead et al. 2004; Lacey et al. 1987) receptors so that excitation could be studied in isolation. The relative contributions of AMPA receptors, NMDA receptors, and GPCRs to transient high-frequency firing in substantia nigra dopamine neurons were then dissected using the application of selective antagonists. These data demonstrated that synaptic activation of both AMPA and NMDA receptors were critical for transient high-frequency firing. Finally, visually guided glutamate ejection onto dopamine neuron dendrites was employed to confirm the role of dendritic AMPA and NMDA receptors (Paquet et al. 1997) in the generation of transient high-frequency activity.

METHODS

Slice preparation

All procedures involving animals were carried out in accordance with the APS’s Guiding Principles in the Care and Use of Animals and were approved by the IACUC of Northwestern University. Brain slices were prepared from (p15-25) male Sprague-Dawley rats. Rats were killed with ketamine and xylazine and perfused via the ascending aorta with 10 –20 ml of ice-cold modified artificial cerebrospinal fluid (ACSF), which contained (in mM) 230 sucrose, 26 NaHCO\(_3\), 2.5 KCl, 1.25 Na\(_2\)HPO\(_4\), 0.5 CaCl\(_2\), 10 MgSO\(_4\), and 10 glucose and was equilibrated with 95% O\(_2\)-5% CO\(_2\). The brain was then rapidly removed, blocked along the midline, glued to the stage of a vibratome (3000; Vibratome, St Louis, MO) and immersed in modified ACSF maintained at \(~0.5–1.5°C\). Sagittal slices containing the substantia nigra were cut at a thickness of 300 \(\mu\)m and then transferred to a holding chamber. Cut slices were then moved into ACSF at room temperature in “traditional” ACSF, which contained (in mM) 126 NaCl, 26 NaHCO\(_3\), 2.5 KCl, 1.25 Na\(_2\)HPO\(_4\), 2 CaCl\(_2\), 2 MgSO\(_4\), and 10 glucose and was equilibrated with 95% O\(_2\)-5% CO\(_2\).

Visualized recording

Individual slices were transferred to a recording chamber that was perfused at 2–4 ml/min with ACSF that more closely mimics rodent brain interstitial fluid (Sanchez-Vives and McCormick 2000). ACSF for recording contained (in mM) 126 NaCl, 26 NaHCO\(_3\), 3 KCl, 1.25 Na\(_2\)HPO\(_4\), 1.6 CaCl\(_2\), 1.5 MgSO\(_4\), and 10 glucose, was equilibrated with 95% O\(_2\)-5% CO\(_2\), and was maintained at \(~37°C\). A \(\times 5\) objective (Olympus, Tokyo) was used to locate the substantia nigra within each slice. A \(\times 40\) water-immersion objective (Zeiss, Oberkochen, Ger-
FIG. 1. Electrophysiological identification of substantia nigra dopamine neurons. A–D: electrophysiological properties of a representative substantia nigra dopamine neuron. The neuron discharged in a slow, single spike pacemaker mode (A) and exhibited broad action potentials of >2 ms duration (B), a characteristic sag in membrane potential in response to hyperpolarizing current injection (C, −150 pA), and strong action potential accommodation in response to depolarizing current injection (D, 300 pA). The dopamine neuron that was physiologically characterized in A–D was filled with biocytin, which was visualized with Alexa Fluor 594 (Ei, red), and was immunoreactive for tyrosine hydroxylase, which was visualized with Cy2 (Eii, green). Eiii: colocalization of the 2 fluorophores within the same neuron is apparent in the merged image (yellow/orange). F: setup of recording and stimulating electrodes in the substantia nigra. STN, subthalamic nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata.

Immunocytochemical detection of tyrosine hydroxylase immunoreactivity in recorded neurons

Immunocytochemical detection of tyrosine hydroxylase was carried out in physiologically characterized neurons to determine whether the classical physiological properties of dopaminergic neurons in the substantia nigra are consistent predictors of dopaminergic phenotype (Grace and Onn 1989; Richards et al. 1997) when applied to the age group of animals and recording techniques used in this study. Putative dopaminergic and nondopaminergic neurons were recorded in the whole cell configuration and filled with biocytin (0.5%). Spontaneous activity and responses to current injection were monitored for <5 min to minimize dialysis of tyrosine hydroxylase. After recording, the slices were fixed in 4% paraformaldehyde overnight at 4°C. Slices were then rinsed several times in phosphate buffered saline (PBS, 0.1 M, pH 7.4) and then placed in cryoprotectant (25% sucrose, 10% glycerol in 0.05 M phosphate buffer, pH 7.4) for 1 h. Slices then were subjected to one to three cycles of rapid freezing in 2-methyl butane (Sigma)/liquid nitrogen followed by thawing in cryoprotectant to maximize penetration of immunoreagents. Slices were then rinsed in PBS, infiltrated with 5% bacto-agar solution and sectioned at 40 μm using a vibratome (Leica, VT1000S, Leica Microsystems, Nussloch, Germany).

All incubations were performed at room temperature on free-floating sections under gentle agitation in PBS containing bovine serum albumin (1%, Jackson Immunoresearch, West Grove, PA), Triton-X100 (0.5%, Sigma), and normal donkey serum (1%, Jackson Immunoresearch). Sections were incubated in mouse anti-tyrosine hydroxylase monoclonal antibody (0.1%; Chemicon, Temecula, CA) overnight. The sections were then rinsed in PBS and incubated in Cy2-conjugated donkey anti-mouse IgG (1%; Jackson Immunoresearch) for 2 h. Sections were then washed in PBS before incubation in streptavidin Alexa Fluor 594 conjugate (0.2%; Invitrogen) for an additional hour. Finally, sections were rinsed and mounted on slides in 50% PBS/50% glycerol and fluorescent images of recorded filled neurons and their immunoreactivity for tyrosine hydroxylase were obtained using confocal imaging (FV-300 confocal microscope, Olympus, Japan).
Synaptic stimulation

Excitatory postsynaptic potentials (EPSPs) were evoked by electrical stimulation (A360 stimulus isolator; World Precision Instruments, Sarasota, FL). Pilot experiments revealed that the stimulation parameters used here evoked stable responses that were not associated with cellular or synaptic plasticity. The stimulating electrode (FHC, Bowdoinham, ME) was placed within the substantia nigra. The stimulating poles were selected from a custom-built matrix comprised of 20 tungsten electrodes. The matrix consisted of four rows of five electrodes (separation: within rows, 240 μm; between rows, 600 μm). Electrode poles that produced large and reliable EPSPs were selected. The duration of each stimulus was 100 ms, and intensities typically ranged between 100 and 600 μA.

Inhibitory postsynaptic potentials were blocked by continuous bath application of GABAA, GABAB, and D2 receptor antagonists. Afferents were then stimulated at 10, 50, and 100 Hz for 200–1,000 ms to generate high-frequency activity. After baseline responses were obtained, ionotropic glutamate receptor, and/or metabotropic glutamate (mGluR) and muscarinic acetylcholine receptor (mAChR) antagonists were applied and responses were retested.

Pressure-pulse application of glutamate

Cell-attached somatic recording and dendritic glutamate ejection pipettes were placed using infragradient contrast imaging. Glutamate [1 mM in a solution containing (in mM) 140 NaCl, 23 glucose, 15 HEPES, 2KCl, 2 MgCl2, 1 CaCl2 – pH 7.2, 300 mosM] was ejected from patch pipettes using pressure pulses (25 pulses at 50 Hz; duration of each pulse: 10 ms; interpulse interval: 10 ms; pressure: 100–200 mbar) that were applied with a Picospritzer III (Parker Hannifin, Cleveland, OH). After recording, neurons were filled with Alexa Fluor 594 hydrazide (Invitrogen) by establishing the whole cell configuration. The extent of glutamate ejection (marked by Alexa Fluor 594 hydrazide in the ejection solution), in relation to the recorded neuron, was assessed by shuttered (VS25 shutter driven by a VMM-D1 driver, Uniblitz, Vincent Associates, Rochester, NY), epifluorescent imaging (UV light source: HBO 100, Zeiss; filter set 31, Zeiss). Image J (National Institutes of Health) was used to control the capture (Psion Teklogix, Mississauga, Canada) of a 30-ms duration image from a CCD camera (IR1000, Dage MTI, Michigan City, MI) in the absence of glutamate ejection or at the end of glutamate ejection.

Drugs

Drugs were bath applied at the following concentrations: 50 μM (+)-2-amino-5-phosphono-pentanoic acid (APV); 1–2 μM CGP55845; 50 μM 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester (CPCCOEt); 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX); 50 μM GYKI133655; 20 μM SR95531; 10 μM sulpiride; and 1 μM scopolamine. All drugs were obtained from Tocris-Cookson (Ellisville, MO).
Analysis

Data were analyzed using Origin 7.5 (Microcal, Northampton, MA) and Igor Pro 5.0 (Wavemetrics, Lake Oswego, OR). Numerical data are presented as means ± SD. Distributions of data are stated numerically as ranges and/or represented graphically with box plots. Paired and unpaired statistical comparisons were made using the nonparametric Wilcoxon’s signed-rank test and Mann-Whitney U test, respectively. For tests in which multiple comparisons were necessary, the calculated P values were adjusted using the Bonferroni correction. A P value of <0.05 was used as the criterion for determining statistically significant differences.

A custom-written routine was used to remove stimulation artifacts produced during the synaptic stimulation experiments. Data (≤1 ms) were deleted for each artifact and then replaced with a straight line that spanned the deleted section. Another routine was used to generate each peristimulus time histogram (PSTH). This routine calculated the time difference between the peak of each action potential (event) and the onset of the stimulation artifact immediately preceding it. Events were separated according to the calculated time difference into 10 2-ms bins. Events were then converted into percentages of the total number of events for each neuron, and the percentages from individual neurons were then averaged to generate a population PSTH for each drug condition.

RESULTS

Dopamine neuron identification

Putative dopamine neurons were visually identified by their location within the substantia nigra and the relatively large size of their cell body (20–30 μm) and proximal

<table>
<thead>
<tr>
<th>TABLE 1. Comparison of spontaneous and evoked activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
</tr>
<tr>
<td>Maximum frequency, Hz</td>
</tr>
<tr>
<td>Mean frequency, Hz</td>
</tr>
<tr>
<td>Number of action potentials</td>
</tr>
<tr>
<td>Time to 1st action potential, ms</td>
</tr>
</tbody>
</table>

Comparison of spontaneous activity to activity evoked by 10-, 50-, and 100-Hz synaptic stimulation for 1 second for 12 substantia nigra dopamine neurons recorded in the perforated (n = 6), whole-cell (n = 3) and cell-attached configurations (n = 3). Data presented as means ± SD, range. *, significantly different from 100 Hz, P < 0.05; †, significantly different from 50 Hz, P < 0.05; ‡, significantly different from 10 Hz, P < 0.05.
dendrites. Recordings from dopamine neurons were confirmed by their characteristic electrophysiological properties, which include slow (1–5 Hz) spontaneous firing (Fig. 1A), prolonged duration (>2 ms) action potentials (Fig. 1B), pronounced sag in the membrane potential response to hyperpolarizing current injection (Fig. 1C), and strong accommodation in response to depolarizing current injection (Fig. 1D). In line with previous studies that employed older animals and sharp microelectrodes (Grace and Onn 1989; Richards et al. 1997), each neuron that exhibited these physiological properties was immunoreactive when tested for tyrosine hydroxylase (Fig. 1E; n = 6). Dopaminergic neurons were therefore readily distinguished from neurons that exhibited the classical electrophysiological properties of GABAergic substantia nigra output neurons (Atherton and Bevan 2005; Grace and Onn 1989; Richards et al. 1997) and were, in each case, immunonegative for tyrosine hydroxylase (n = 5; see supplementary figure and table).

To examine the effects of excitatory synaptic inputs in isolation, GABA_A, GABA_B, and D_2 receptors were continuously blocked by selective antagonists. Local stimulation (Fig. 1F) at 10–100 Hz generated transient high-frequency activity in neurons recorded in perforated (Fig. 2A; n = 6), whole cell (Fig. 2B; n = 3), or cell-attached (Fig. 2C; n = 3) configurations. Synaptically evoked maximum/mean firing rates and the number of evoked action potentials were significantly greater than mean spontaneous activity (Table 1 and Fig. 2E). Latencies between the onset of 50 to 100 Hz synaptic stimulation and the first evoked action potential were significantly less than the latency associated with 10 Hz stimulation (Table 1). Thus synaptic input, in contrast to somatic current injection (Grace and Bunney 1983a; Kita et al. 1986; Richards et al. 1997), reliably evokes robust transient high-frequency activity in substantia nigra dopamine neurons.

Local electrical stimulation generates transient high-frequency firing in dopamine neurons

1 The online version of this article contains supplemental data.
evoked activity. Subsequent application of the selective AMPA-kainate receptor antagonist DNQX caused a marked reduction in the frequency of transient high-frequency activity and greatly altered the characteristic pattern of action potential amplitude, presumably due to the filtering characteristics of resistances consistently exhibited a similar pattern of action potential during spontaneous activity in vivo (Fig. 3). Recordings with higher series resistances revealed that local synaptic stimulation evoked transient high-frequency activity that was similar in form to that observed in vivo (Chergui et al. 1993, 1994a; Grace and Bunney 1983a; Overton and Clark 1992; Paladini and Tepper 1999; Smith and Grace 1992). Transient high-frequency or “burst” firing in vivo is typically associated with a progressive reduction in the frequency of action potential generation, a progressive elevation in action potential threshold, a progressive reduction in action potential amplitude, and a progressive increase in action potential duration. Detailed analysis of six representative neurons recorded with low series resistances in the perforated (n = 3) and whole cell (n = 3) configurations revealed that local synaptic stimulation evoked transient high-frequency activity that was similar in form to that reported in vivo (Fig. 3). Recordings with higher series resistances consistently exhibited a similar pattern of action potential generation and action potential threshold and duration but did not exhibit a progressive reduction in spike amplitude, presumably due to the filtering characteristics of high resistance recording.

**Synaptically evoked transient high-frequency activity is similar in form to that observed in vivo**

High-frequency activity of dopamine neurons that has been observed in previous in vitro studies (Johnson et al. 1992; Li et al. 1996) exhibited a pattern of action potential generation that was distinct from that observed in vivo (Chergui et al. 1993, 1994a; Grace and Bunney 1983a; Overton and Clark 1992; Paladini and Tepper 1999; Smith and Grace 1992). Transient high-frequency or “burst” firing in vivo is typically associated with a progressive reduction in the frequency of action potential generation, a progressive elevation in action potential threshold, a progressive reduction in action potential amplitude, and a progressive increase in action potential duration. Detailed analysis of six representative neurons recorded with low series resistances in the perforated (n = 3) and whole cell (n = 3) configurations revealed that local synaptic stimulation evoked transient high-frequency activity that was similar in form to that reported in vivo (Fig. 3). Recordings with higher series resistances consistently exhibited a similar pattern of action potential generation and action potential threshold and duration but did not exhibit a progressive reduction in spike amplitude, presumably due to the filtering characteristics of high resistance recording.

**Blockade of AMPA and NMDA receptors eliminates transient high-frequency activity**

Application of the selective AMPA-kainate receptor antagonist DNQX caused a marked reduction in the frequency of evoked activity and greatly altered the characteristic pattern of evoked activity. Subsequent application of the selective NMDA receptor antagonist APV completely abolished the effects of synaptic stimulation (Fig. 4 and Table 2). Furthermore, in the absence of AMPA-kainate and NMDA receptor activation, local (50–100 Hz) stimulation did not significantly affect firing or the magnitude of action potential afterhyperpolarization compared with spontaneous activity (Fig. 4 and Tables 2 and 3). These data suggest that GPCR-mediated reductions in action potential afterhyperpolarization do not contribute greatly to transient high-frequency firing in vitro.

**Comparison of action potential afterhyperpolarization**

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Control</th>
<th>DNQX</th>
<th>APV, DNQX</th>
<th>Spontaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 Hz</td>
<td>−61.7 ± 6.3</td>
<td>−67.3 ± 7.1*</td>
<td>−68.3 ± 8.5*</td>
<td>−65.1 ± 6.6*</td>
</tr>
<tr>
<td>100 Hz</td>
<td>−59.8 ± 5.9</td>
<td>−65.7 ± 6.8*</td>
<td>−68.3 ± 8.5*</td>
<td>−65.1 ± 6.6*</td>
</tr>
</tbody>
</table>

Comparison of action potential afterhyperpolarization during synaptic stimulation at 50–100 Hz under control conditions and in the presence of GYKI/DNQX and APV and action potential afterhyperpolarization during spontaneous activity in 7 neurons in which DNQX was applied before APV and 7 neurons in which APV was applied before GYKI/DNQX. During the period of electrical stimulation, action potential afterhyperpolarization in the presence of GYKI/DNQX and APV was not significantly different from the afterhyperpolarization associated with spontaneous activity, *P < 0.05.
by joint application of APV and GYKI/DNQX, the magnitudes of action potential afterhyperpolarizations observed during synaptic stimulation (50–100 Hz) were not different from afterhyperpolarizations associated with spontaneous activity (Table 3).

In several cells, GYKI and DNQX were applied sequentially to assess a possible role for kainate receptors in high-frequency firing. The number of action potentials evoked in APV and GYKI during 50 to 100 Hz stimulation (2.2 ± 0.6) and those evoked in APV, GYKI, and DNQX during 50 to 100 Hz stimulation (2.1 ± 0.8) were not significantly different from each other or spontaneous activity (2.5 ± 0.6; n = 10, P > 0.05). Furthermore, the application of DNQX in the presence of the selective AMPA receptor antagonist GYKI had no further effect on action potential afterhyperpolarization (action potential afterhyperpolarization during 50 to 100 Hz stimulation in APV and GYKI: −69.7 ± 4.4 mV; action potential afterhyperpolarization in APV, GYKI, and DNQX during 50 to 100 Hz stimulation: −70.0 ± 6.2 mV; n = 8, P > 0.05). Thus AMPA rather than kainate receptors underlie the effect of DNQX on the evoked activity described here. Indeed GYKI-resistant, DNQX-sensitive kainate receptor-mediated EPSPs or effects on activity were not observed (Fig. 5). Because application of DNQX in the presence of the selective AMPA receptor antagonist GYKI had no further effect on evoked activity, these data were pooled in Tables 2 and 3. Taken together, these data suggest that synaptic activation of ionotropic AMPA and NMDA receptors are sufficient for transient high-frequency activity in vitro.

Closer inspection of evoked activity (Fig. 6) revealed that AMPA receptor-mediated EPSPs, presumably due to the relatively rapid nature of their underlying conductance, are more distinct than those generated by NMDA receptors. Furthermore, analysis of composite PSTHs revealed that blockade of AMPA receptor-mediated EPSPs abolished the precise temporal relationship between evoked activity and stimulation (Fig. 6). Interestingly, NMDA receptor-mediated EPSPs in isolation could occasionally generate action potential doublets of high instantaneous frequency.

**FIG. 5.** Synaptically evoked transient high-frequency activity is mediated through AMPA and NMDA receptors. Fifty (A) and 100 Hz (B) electrical stimulation evoked transient high-frequency activity in a substantia nigra dopamine neuron recorded in the perforated-patch configuration. The characteristic discharge pattern was disrupted by application of APV. In the absence of NMDA receptor-mediated transmission, AMPA-kainate receptor-mediated excitation evoked lower frequency activity. Application of APV and GYKI completely abolished the synaptic responses indicating that they were mediated by NMDA and AMPA receptors, respectively. Application of DNQX after GYKI had no further effect on firing (not illustrated). Neurons in Ai and Bi are the same. Aii and Bii illustrate the effects of ionotropic glutamate receptor antagonists on evoked activity in the sample population (n = 11).
previously been reported to flow during subthreshold pacemaker activity (Wolfart and Roepert 2002). In six neurons tested, the peak AMPA receptor-mediated current was $-163.0 \pm 67.9$ pA and over 1 s of stimulation carried $-29.8 \pm 16.3$ pC of charge, whereas NMDA receptor-mediated currents peaked at $-120.4 \pm 67.5$ pA and carried $-38.2 \pm 31.2$ pC of charge.

**Glutamatergic and cholinergic GPCRs reduce the intensity of synaptically evoked transient high-frequency activity**

The experiments described in the preceding text indicate that synaptic activation of ionotropic glutamate receptors exerts a powerful influence on the firing pattern of substantia nigra dopamine neurons. To further address the relative contribution of glutamatergic and cholinergic GPCRs, evoked activity was also compared under control conditions and in the presence of the mGlu1 receptor antagonist CPCO0Et and the broad-spectrum muscarinic receptor antagonist scopolamine in six neurons. Application of these GPCR antagonists significantly increased the intensity of evoked activity for all firing parameters at 50 Hz stimulation and for mean evoked activity at 100 Hz stimulation (Fig. 7). These data suggest that transient high-frequency synaptic activity activates GPCRs in a manner that moderately attenuates rather than facilitates or triggers high-frequency activity in dopamine neurons.

**Dendritic application of glutamate evokes transient high-frequency activity through the activation of AMPA and NMDA receptors**

Given the specialized integrative properties of dopamine neuron dendrites, pressure-pulse application of glutamate (in the presence of antagonists of GABA$_A$, GABA$_B$, D$_2$, and mGlu$_1$, receptors) was used to determine whether activation of dendritic ionotropic glutamate receptors (Paquet et al. 1997) was sufficient to evoke transient high-frequency activity. The pressure-pulse pipette was first placed in the vicinity of a proximal dendrite of the target dopamine neuron using infrared gradient contrast imaging. To minimize the perturbation of intracellular processes and ionic gradients, physiological responses were assessed in the cell-attached configuration (Fig. 8Ai). As for synaptically evoked activity, blockade of AMPA (n = 4 neurons) or NMDA (n = 4 neurons) receptors greatly attenuated activity evoked by 25 10 ms, 100–200 mbar pressure-pulses of glutamate delivered at 50 Hz in each neuron that was tested (Fig. 8 Ai and Bi–ii). The attenuation of evoked activity was partially reversed on removal of the receptor antagonists from the bathing medium. After characterization of physiological responses, the dendritic morphology was verified through establishment of the whole cell configuration with an Alexa Fluor 594-filled recording pipette (Fig. 8Ai). Fluorescent imaging of Alexa Fluor 594 in each recorded neuron during the ejection of glutamate and Alexa Fluor 594 from an ejection pipette confirmed that Alexa Fluor 594, and therefore presumably glutamate, were relatively restricted to a dendrite in each case (Fig. 8Aii–Aiii).

**DISCUSSION**

**Local synaptic stimulation evokes transient high-frequency activity in substantia nigra dopamine neurons**

Although the mechanisms underlying transient high-frequency or “burst” firing of substantia nigra dopamine neurons...
have been the subject of much debate, the conditions and parameters necessary to reliably evoke and thus study such activity in vitro have not been thoroughly documented. Because local electrical stimulation is not expected to reproduce the timing of excitation and inhibition that occurs during transient high-frequency activity in vivo and electrical stimulation in vitro can generate powerful IPSPs that inhibit activity, GABA receptor-mediated (Gulacsi et al. 2003; Hausser and Yung 1994; Iribe et al. 1999) and dopamine autoreceptor mediated (Beckstead et al. 2004; Lacey et al. 1987) synaptic responses were blocked with selective antagonists. Under these conditions, local stimulation at 50–100 Hz but not 10 Hz rapidly (within \( \leq 50 \) ms) generated transient high-frequency activity that was similar in form to “burst” firing in awake or anesthetized animals (Chergui et al. 1993; Grace and Bunney 1983a,b; Hyland et al. 2002; Overton and Clark 1992; Paladini and Tepper 1999; Schultz 2002; Smith and Grace 1992; Wilson et al. 1977).

Similar patterns of activity were evoked when using noninvasive (cell-attached), moderately invasive (perforated), and invasive (whole cell) recording configurations. The robustness of the pattern and mechanisms underlying evoked activity under different recording conditions suggest that artifactual reductions in afterhyperpolarization, dialysis with an alien \( Ca^{2+} \) buffer (Neher and Augustine 1992; Velumian and Carlen 1999; Velumian et al. 1997; Zhang et al. 1994, 1995), and disruption of GPCR signaling cascades (Brown et al. 1989; Suh et al. 2004) contributed minimally to the pattern of evoked activity.

**Ionotropic glutamate receptors underlie evoked transient high-frequency activity**

Although a variety of synaptic receptors may contribute to transient high-frequency activity, the contribution of AMPA and NMDA receptors was clearly dominant. Interestingly, simultaneous activation of both classes of receptor was essential for this form of activity in vitro. Blockade of either class of receptor not only reduced the number and frequency of action potentials that were evoked but also altered the characteristic temporal pattern and action potential morphology associated with high-frequency discharge in dopamine neurons. These observations are at odds with in vivo studies in which transient high-frequency discharge was blocked by NMDA but not AMPA receptor antagonists (Chergui et al. 1993, 1994a; Overton and Clark 1992; Tong et al. 1996). One possible reason for the discrepancy is that the in vitro recordings were made in young rats and the in vivo studies were made in more mature animals in which NMDA receptor-mediated transmission is dominant. However, in general, the ratio of AMPA to NMDA receptors tends to be conserved (Hohnke et al. 2000; Myme et al. 2003) or increase (Arsenault and Zhang 2006; Ye et al. 2005) with age. Therefore another possibility is that application of AMPA receptor antagonists in vivo exerted polysynaptic effects, which partly compensated for the reduced excitation of substantia nigra dopamine neurons, e.g., reduced AMPA receptor-mediated drive of GABAergic substantia nigra neurons may have led to the disinhibition of substantia nigra dopamine neurons (Celada et al. 1999; Grace and Bunney...
GPCRs do not underlie transient high-frequency activity in vitro

Despite prolonged (1 s), tetanic (≤100 Hz) stimulation of afferent fibers, which led to the release of glutamate (as evidenced by the activation of ionotropic glutamatergic receptors) and presumably other transmitters (e.g., acetylcholine), no evidence was found for a GPCR mediated reduction in action potential afterhyperpolarization and/or GPCR mediated excitation through activation of a cation current (Bengtson et al. 2004; Grillner and Mercuri 2002; Lacey et al. 1990; Scroggs et al. 2001; Shen and Johnson 1997). On the contrary, GPCR antagonists moderately increased the frequency of evoked discharge. These data support the findings of Williams and colleagues that transient activation of GPCRs evokes a slow IPSP in substantia nigra dopamine neurons that contributes to the moderation/termination of high-frequency discharge (Fiorillo and Williams 1998, 2000; Morikawa and Williams 2003; Paladini and Williams 2004). More prolonged activation of GPCRs in vivo could, however, through desensitization of the signaling cascades underlying the IPSP, facilitate, through reductions in afterhyperpolarization and/or activation of cation current (Fiorillo and Williams 1998, 2000; Morikawa and Williams 2003; Paladini and Williams 2004), the ability of AMPA and NMDA receptor-mediated excitation to evoke high-frequency activity.

Mechanisms underlying transient high-frequency activity of substantia nigra dopamine neurons

This study provides definitive evidence that ionotropic glutamate receptor mediated synaptic transmission can evoke transient high-frequency activity in substantia nigra dopamine neurons without a GPCR-mediated reduction in single-spike afterhyperpolarization. Indeed, comparison of the magnitude of AMPA and NMDA receptor-mediated currents (Mereu et al. 1997; this study) and the smaller current underlying single-spike afterhyperpolarization (Wolfart and Roeper 2002) suggests that synaptic influences could transiently overwhelm the afterhyperpolarization.

It is also likely that AMPA and NMDA receptor-mediated synaptic currents engage postsynaptic dendritic voltage-dependent ion channels in a manner that contributes to high-frequency activity. AMPA receptor-mediated currents may contribute solely by increasing the level of dendritic depolarization and thus increasing the conductance of the voltage-dependent NMDA receptor (Blitz and Regehr 2003; Gauck and Jaeger 2003; Harsch and Robinson 2000). Although the relatively voltage-independent AMPA receptor has been suggested to underlie a current similar to current steps injected via somatic recording pipettes (Kuznetsov et al. 2006), synaptically evoked AMPA receptor-mediated currents may differ greatly in their kinetics and sites of action. Thus the rapid time course of AMPA receptor-mediated currents and their dendritic location may contribute to the formation of dendritic action potentials (cf. Chen et al. 1997; Gasparini and Magee 2006; Golding and Spruston 1998; Hanson et al. 2004; Jarsky et al. 2005; Martina et al. 2000). Indeed, dendritic outside-outside patch recordings confirm that voltage-dependent Na+ channels are expressed at high density in the dendrites of substantia nigra dopamine neurons (Haussler et al. 1995), and these channels support both active back propagation of action potentials (Haussler et al. 1995) and possibly dendritic action potential initiation (Nedergaard and Hounsgaard 1996). Furthermore, dopamine neurons possess morphological properties that may favor dendritic spike initiation (Haussler et al. 1995; Grace and Bunney 1983b; Tepper et al. 1987; Vetter et al. 2001). Although several studies suggest that autonomously and synaptically generated action potentials are typically initiated in the proximal axon (Clark et al. 2005; Khaliq and Raman 2006; Meeks et al. 2005; Palmer and Stuart 2006), it has been established in several classes of neuron that temporally and spatially clustered excitatory synaptic inputs can shift the site of action potential initiation to dendrites (Chen et al. 1997; Gasparini and Magee 2006; Golding and Spruston 1998; Hanson et al. 2004; Jarsky et al. 2005; Martina et al. 2000).

The voltage dependence of the NMDA receptor has also been suggested to be critical for boosting the contribution of high-frequency dendritic Ca2+-dependent oscillations (Kusnetsov et al. 2006). However, purely NMDA receptor mediated transient high-frequency activity was not observed in response...
to synaptic stimulation. Interestingly, however, action potential doublets of high instantaneous frequency were observed in the presence of NMDA receptor-mediated but not AMPA receptor-mediated excitation. Under these conditions, we propose that action potential mediated unblock of the NMDA receptor (cf. Kampa et al. 2004) and the relatively slow time course of NMDA receptor kinetics in dopamine neurons (Gotz et al. 1997) may contribute to the occurrence of a second action potential with brief delay. An additional possibility is that the combination of action potentials and NMDA receptor activation generates sufficient depolarization for the powerful recruitment of postsynaptic Ca\textsubscript{\textit{\textit{V}}} channels (Kusnetsov et al. 2006; Ping and Shepard 1999).

**Functional considerations**

The rapid response of substantia nigra dopamine neurons to rewards and salient stimuli (Hyland et al. 2002; Morris et al. 2004; Schultz 2002) suggests that the synaptic pathways and receptors underlying such activity must also be rapid in their time course. It is therefore perhaps not surprising that ionotropic glutamate receptors rather than GPCRs are critical for high-frequency activity. In this context, basal activation of GPCRs in vivo (e.g., Miller and Blaha 2005), which underlie reductions in afterhyperpolarization and cation currents would therefore be predicted to facilitate rather than trigger high-frequency activity.

The capability of AMPA and NMDA receptor activation to generate transient high-frequency activity in dopamine neurons suggests that plasticity in the number, distribution and/or subunit composition of AMPA and NMDA receptors induced by specific patterns of pre- and postsynaptic activity (Bonci and Malenka 1999; Liu et al. 2005; Overton et al. 1999; Thomas et al. 2000) or drugs of abuse (Jones et al. 2000; Liu et al. 2005; Schilstrom et al. 2006; Ungless et al. 2001; Zhang et al. 1994) could modify excitatory synaptic integration in dopamine neurons, their activity pattern in vivo, and their ultimate influence on behavior. Such plasticity could underlie natural reward related learning and/or behavioral sensitization/addictive behavior generated by drugs of abuse. In general, an upregulation of functional AMPA receptors underlies long-term potentiation in dopamine neurons although NMDA receptors are critical for the induction of plasticity (Bonci and Malenka 1999; Liu et al. 2005; Overton et al. 1999; Ungless et al. 2001; Zhang et al. 1997). Drugs of abuse can also reduce GABAergic synaptic transmission (Liu et al. 2005) and inhibitory glutamatergic GPCR signaling (Paladini et al. 2001), which together may further enhance the transient high-frequency activity of dopamine neurons generated by the synaptic activation of ionotropic glutamate receptors and potentiation of their excitatory input.

Although transient high-frequency activity is critical for the function of dopamine neurons, the cellular processes underlying and engaged by such activity may also contribute to their demise in Parkinson’s disease. Influx of Ca\textsuperscript{2+} via synaptic receptors and Ca\textsubscript{\textit{\textit{V}}} channels could contribute to mitochondrial dysfunction, to which these neurons appear particularly susceptible (Chinopoulos and Adam-Vizi 2006; Greenamyre and Hasting 2004; Jacquard et al. 2006; Kupsch et al. 1996; Vercesi et al. 2006).

**Acknowledgments**

We thank C. Wilson, H. Kita, and D. J. Surmeier for constructive comments. We are particularly appreciative of Dr. Stephen Kitai for support and scientific contribution to this research.

**Grants**

This research was supported by National Institutes of Neurological Disorders and Stroke Grants NS-020702, NS-047085, and NS-041280 and a National Parkinson Disease Foundation grant to M. D. Bevan and a Picower Foundation Grant to D. J. Surmeier. S. N. Blythe was supported by National Institutes of Health Grants AG-020418 and MH-067564 and is a graduate student of the Northwestern University Interdepartmental Neuroscience Program.

**References**


Kupsch A, Sautter J, Schwarz J, Riederer P, Gerlach M, Oertel WH. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in non-human primates is antagonized by pretreatment with nimodipine at the nigral, but not at the striatal level. *Brain Res* 741: 185–196, 1996.


