EVERY BASAL GANGLIA NUCLEUS contains autonomously active neurons, and in every nucleus save the striatum, such neurons predominate (Surmeier et al. 2005). Intrinsically oscillating neurons can be represented by phase models, where the state of the neuron is reduced to a single number, the phase, that denotes what fraction of its oscillation cycle the cell has traversed (Galán 2009; Gutkin et al. 2005; Rinzel and Ermentrout 1998; Winfree 2001). In phase models, synaptic inputs act by advancing or delaying the neuron’s phase, thereby shortening or lengthening the time to the next spike. The response to a synaptic input usually depends on the phase at which the input arrives, and the relationship between the phase shift induced by an input and phase at which it arrived is called the phase response curve (PRC). For cells in simple circuits receiving a small number of inputs, the PRC for each input could be measured individually and used to understand the behavior of the network. For neurons like those in the basal ganglia, which receive thousands of diverse synaptic inputs, it may still be possible to apply this method by using the infinitesimal PRC (iPRC), the rate at which an applied current changes a cell’s phase as a function of input phase, to predict the response to arbitrary synaptic currents. The iPRC can be estimated from the response of a cell to small, brief current pulses (Bennett and Wilson 1998; Galán et al. 2005; Mancilla et al. 2007; Phoka et al. 2010; Reyes and Fetz 1993a, 1993b; Stiefel et al. 2008; Tateno and Robinson 2007; Tsubo et al. 2007) or simulated conductance changes (Netoff et al. 2005a, 2005b; Preyer and Butera 2005; Sieling et al. 2009), and, combined with knowledge of synaptic currents, it should in principle be possible to predict the cell’s response to its combined input from all other cells in the network. Phase models are especially suited for studying the conditions required for network synchrony (e.g., Ermentrout 1996; Hansel et al. 1995; van Vreeswijk et al. 1994), and this approach to modeling the basal ganglia may be useful in explaining why its neurons tend to fire asynchronously under normal conditions but participate in synchronized oscillations in Parkinson’s disease (Rivlin-Etzion et al. 2010) and how inputs arriving in the striatum and subthalamic nucleus affect basal ganglia output.

Phase models are extremely simple, making them analytically tractable and facilitating the study of the collective behavior of large neural populations. Yet, they may be so simple that they fail to capture the behavior of neurons with sufficient accuracy, producing incorrect inferences about network dynamics. For example, the iPRC may depend on the firing rate (Phoka et al. 2010), since at least some intrinsic conductances cannot be expected to operate on faster time-scales as the interspike interval (ISI) shortens. Furthermore, the iPRC may not accurately describe the response to larger inputs, perhaps not even to inputs as large as a unitary synaptic potential (Acker et al. 2003; Netoff et al. 2005b). In addition, synaptic potentials are generated by conductance changes, often at electrotonically distant sites on dendrites, whose effects may not be well represented by the effect of current injected at the soma (Goldberg et al. 2007; Schultheiss et al. 2010). Finally, inputs activating NMDA-type glutamate receptors (NMDARs) can be expected to pose special problems for the iPRC description, because the NMDAR conductance is prolonged and is a nonlinear function of membrane potential. We addressed these issues experimentally in the subthalamic nucleus (STN). Excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the internal capsule were used to measure synaptic iPRCs; in most cells, iPRCs were also estimated from the response to injected current pulses. We examined the effect of firing rate on iPRC structure by comparing iPRCs measured in STN cells firing at a wide range of intrinsic rates. To assess the accuracy of the iPRC prediction with stronger inputs, we looked for systematic changes in iPRC
shape with increasing EPSP size. To determine whether systematically injected current had the same effect on spike timing as synaptically generated conductance changes, we compared iPRCs measured with EPSPs with those measured using current pulses and evaluated the effect of NMDAR antagonists on both kinds of iPRC. Although some aspects of our results are necessarily specific to the STN, our data and our approach should illuminate the effectiveness and limitations of phase models in other cell types.

MATERIALS AND METHODS

All experimental procedures followed National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of the University of Texas San Antonio.

Electrophysiological recording. To make brain slices containing the subthalamic nucleus, 15- to 23-day-old Sprague-Dawley rats of either sex were deeply anesthetized with isoflurane and perfused transcardially with ice-cold low-sodium artificial cerebrospinal fluid (ACSF) containing (in mM) 230 sucrose, 2.5 KCl, 1.25 NaH2PO4, 0.5 CaCl2, 10 MgSO4, 10 d-glucose, and 26 NaHCO3. Brains were rapidly removed and sectioned at 300 μm with a vibrating microtome; the plane of section was rotated ~15° off the parasagittal plane to maximize the intact length of corticostriatal fibers in the internal capsule. Slices were collected into normal ACSF containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 2 MgSO4, 10 d-glucose, and 26 NaHCO3. Slices were stored at room temperature while ACSF was bubbled continuously with a 95%-5% O2-CO2 mixture. For recording, slices were submerged and superfused continuously with oxygenated ACSF heated to 31–33°C. Recording pipettes were fabricated with a Flaming-Brown pipette puller (model P-97; Sutter Instruments, Novato, CA) to obtain tip diameters of 1–3 μm (electrode resistance 2–6 MΩ). Cells were visualized with an Olympus BX50WI microscope equipped with a ×40 water-immersion objective and differential interference contrast optics.

All cells were recorded with the perforated-patch technique using gramicidin. In addition to gramicidin, the pipette solution contained (in mM) 135 K-methylsulfate, 10 HEPES, 5 EGTA, and 2 MgCl2. We used two different concentrations of gramicidin-D, depending on whether the pipette solution was filtered before or after addition of gramicidin. If the solution was filtered after addition of gramicidin, we used 50–100 g/ml. Electrodes were tip-filled with gramicidin-free solution and backfilled with solution containing gramicidin. Data were recorded with a MultiClamp 700B amplifier (Molecular Devices, Palo Alto, CA), low-pass filtered at 4 kHz, and acquired at 10 kHz with an ITC-18 digital-to-analog converter (Instrutech, Port Washington, NY). Membrane potential was corrected for postrecording voltage offset. Synaptic responses were evoked with bipolar tungsten microelectrodes (tip spacing 250–500 μm) placed in the internal capsule, typically >1 mm rostral and dorsal to the STN; stimuli were 100-μs pulses, 0.1–3 mA. All recordings were made in the presence of the GABA\textsubscript{A} channel blocker picrotoxin (150 μM) and the GABA\textsubscript{B} receptor antagonist CGP-55845 (1 μM). In addition, the NMDAR antagonist D,L-2-amino-5-phosphonovaleric acid (APV) was used in some experiments. Picrotoxin and APV were obtained from Sigma (St. Louis, MO); CGP-55845 was obtained from Tocris Bioscience (Ellisville, MO).

Data analysis. Analysis was performed using custom algorithms implemented in Mathematica (Wolfram Research). We defined the time of a spike as the time during the action potential rising phase at which the rate of rise is maximal. Spike threshold was defined as the potential at which the second derivative of the voltage trajectory approaching a spike reached 50 mV/m\textsuperscript{-2}. PRCs give the phase shift induced by a stimulus as a function of the input phase at which the stimulus was delivered (see RESULTS for examples illustrating how PRCs were measured experimentally). Phase shift is the change in the duration of the ISI relative to the duration of unstimulated ISIs; input phase is the fraction of the unstimulated ISI at which the stimulus arrives, i.e., the stimulus time relative to the time of the last spike divided by the unstimulated ISI duration (Rinzel and Ermentrout 1998). Measurement of both input phase and phase shift depends on an estimate of what the ISI would have been without the stimulus, which we call the “base ISI.” Traditionally, the average spontaneous ISI is used as the estimate of the ISI without the stimulus. This is a reasonable choice, but spontaneous ISIs are variable, and the average spontaneous ISI is the best estimate of what the ISI would have been without the stimulus only if we had no other information to use. However, for a given stimulated ISI, we do have some additional information about its unstimulated duration: we know that it would have been at least as long as the input time, i.e., the stimulus time relative to the most recent spike. Instead of using the average of all spontaneous ISIs as our estimator of the unstimulated ISI, we use the average only of spontaneous ISIs longer than the input time; this avoids some artifacts associated with empirical PRC estimation (Polhamus et al. 2012). Furthermore, the pool of spontaneous ISIs used to compute this average for a given stimulated ISI is limited to those occurring within 30 s of the stimulus, to account for the effects of any slow nonstationarities in the spontaneous ISI distribution. On trials with very late input times, there may be few spontaneous ISIs within this pool from which to compute the base ISI. If the number of available spontaneous ISIs longer than the input time is less than 10, the base ISI is instead computed from a Gaussian distribution whose mean and standard deviation are matched to that of the local spontaneous ISIs; the base ISI is then the average of this distribution over values greater than the input time.

Making measurements on EPSPs requires isolation of the EPSP waveform from ongoing membrane potential changes that result from the autonomous oscillatory activity of the cell. To do this, we took ISIs containing evoked EPSPs and subtracted from them the average spontaneous ISI trajectory aligned to the time of the first spike of the ISI. This method is imperfect because the depolarization caused by the EPSP may interact with intrinsic voltage-dependent conductances to produce additional currents that are not removed by subtraction of the spontaneous ISI trajectory aligned to the time of the first spike of the ISI. Furthermore, subthalamic neurons exhibit a near-zero slope conductance over a wide range of membrane potentials due to a balance between the negative slope persistent Na\textsuperscript{+} conductance and more conventional positive slope conductances (Farr\textquotesingle et al. 2010). Thus, by confining our measurements to EPSPs arriving 20–50% through the autonomous ISI, we obtained synaptic potentials that were largely confined to the zero-slope region of the STN cell’s current-voltage (I-V) curve and thereby minimized the contribution of intrinsic membrane currents to our measurements of EPSP amplitude. EPSP measurements obtained this way were nearly independent of phase, and the subtracted waveforms ended in a near-flat trajectory, suggesting that effects of intrinsic voltage-dependent conductances had been largely removed by the subtraction. We averaged these subtracted waveforms (aligned to the time of the stimulus) and examined the first and second time derivatives of this average waveform to judge when the synchronically driven depolarization was complete (i.e., the end time of the EPSP). Ideally, this is when the first derivative has decayed to zero (i.e., when the cell has returned to the rate of depolarization seen in spontaneous ISIs at that time), but the first derivative did not always decay to zero. In such cases, a fit of the experimental waveform was used to select an estimate of the EPSP end time. Initially, this analysis was performed only on trials in which the stimulus artifact came at least 10 ms after the first spike of the stimulated ISI but at least 25 ms before the second spike of the ISIs. This allowed us to identify the EPSP end time and EPSP onset. Using that information, we made measurements on all EPSPs with onset times at least 3 ms after the preceding spike and end times at least 5 ms before the subsequent spike. EPSP amplitude was defined as the membrane potential reached...
at the EPSP end time, relative to the prestimulus potential. The EPSP latency relative to the electrical shock that evoked it was measured from the time at which the EPSP had achieved 50% of its final amplitude. We used this latency to define the input time for an EPSP on a given trial as the time of the stimulus plus the EPSP latency minus the time of the most recent spike. The input phase was then defined as the input time divided by the base ISI duration for that trial. The final values for these measurements were derived from the average waveform of EPSPs arriving at input phases of 0.2–0.5 (i.e., at 20–50% of the unstimulated ISI duration). However, when assessing the effect of membrane potential on EPSP amplitude (RESULTS, Synaptic currents underlying the response to stimulation), we considered all EPSPs that met the minimum latency criteria (i.e., EPSP onset 3 ms after preceding spike and EPSP end time 5 ms before subsequent spike). To combine data from multiple cells (with different EPSP amplitudes) for this analysis, we normalized the individual EPSP amplitudes by their average value at a reference potential of −57 mV; that value, in turn, was derived from the voltage-EPSP size relation for each cell smoothed with a Gaussian filter with a 1-mV standard deviation.

Synaptic iPRCs were estimated by dividing the total phase shift by the EPSP amplitude to give the normalized phase shift per millivolt. On some trials, a spike was fired before the EPSP end time; in such cases, the phase shift was normalized by the fraction of the EPSP amplitude falling within the stimulated ISI. That fractional amplitude was determined from the average EPSP waveform: the time of the spike relative to the stimulus artifact was used to determine where in the average EPSP waveform the spike occurred; the membrane potential at that point in the average EPSP waveform defined the fractional EPSP amplitude. Although our iPRCs did include trials with partial EPSPs occurring at the end of the ISI, they did not include partial EPSPs at the beginning of the ISI, i.e., we did not include trials in which the EPSP was initiated during the preceding ISI but would have continued into the next ISI based on our estimation of the EPSP end time. In other words, the stimulated ISI always included the EPSP onset even if it did not always contain the EPSP end time.

Input time for current pulse stimuli was defined as the time halfway through the pulse, i.e., the current pulse onset time plus one-half of the current pulse duration. Current pulse iPRCs were estimated by dividing the total phase shift by the membrane potential change caused by the current pulse. That membrane potential change was measured using a method similar to our approach to measuring EPSP amplitude: average spontaneous ISI waveforms were subtracted from waveforms containing current pulses, and these difference waveforms were averaged, including only those with input phases of 0.2–0.5. The membrane potential change was measured 5 ms after the end of the current pulse. In most cells, both hyperpolarizing and depolarizing current pulses were used to measure iPRCs, always of equal duration and equal magnitude. In these cases, the membrane potential changes for hyperpolarizing and depolarizing pulses were measured separately, and the absolute values of those changes were averaged to give the magnitude of membrane potential change used for PRC normalization. As with EPSPs, trials in which a spike was triggered during the current pulse were normalized with a fraction of the total measured magnitude. We used several different current pulse durations, including pulse amplitudes of ±25 pA (n = 5), ±30 pA (n = 2), ±40 pA (n = 1), ±50 pA (n = 9), ±75 pA (n = 27), and ±100 pA (n = 16). We also used 1-ms pulses (n = 1), 3-ms pulses (n = 3), and 5-ms pulses (n = 2), all ±20 pA.

Smooth iPRCs are often extracted from noisy data by fitting them with a small number of Fourier modes (e.g., Galán et al. 2005) or polynomial terms (e.g., Netoff et al. 2005a). Because we often could not get good fits to our data with simple functions, we chose to smooth iPRC data with a Gaussian filter with a width measured by standard deviation, of 0.05 phase units; all subsequent analysis was performed on these smoothed curves. To compute iPRC statistics (mean, standard deviation, median, and other quantiles) from a population of individual iPRCs, each smoothed iPRC was sampled at 0.01 phase intervals by using interpolation between the smoothed data points; the desired statistic could then be computed at each sampled phase. The range of sampled phases was limited by the shortest individual iPRCs in the population: the earliest sampled phase was the maximum of the earliest phases in each iPRC, and the latest sampled phase was the minimum of the latest phases in each iPRC. Statistical comparisons on iPRCs are complicated by their phase dependence: for example, two populations may differ at some phases but not others. We dealt with this problem by dividing smoothed iPRCs into relatively narrow bins (0.02 phase units) and calculating the average value for each curve within each bin; statistical tests were then repeated on each bin. This introduced a problem with multiple comparisons in that a comparison of two populations of iPRCs became 41–43 separate tests. We used the Bonferroni correction for multiple comparisons, which divides the target type I error criterion (P = 0.05) by the number of tests, yielding a significance criterion of P = 0.0012. However, this criterion is too stringent, mainly because each comparison is not truly independent (the values in neighboring bins tend to be correlated). For example, if a test shows a significant effect at the P < 0.05 level for 10 consecutive bins but no bin meets the Bonferroni-corrected criterion, that difference is probably real. To get around this, we report when a difference is significant at the P = 0.05 level over consecutive bins as a well as when the Bonferroni-corrected criterion is met. If a test is significant at the P < 0.05 level in multiple consecutive bins but never reaches the more stringent criterion, we divide the phase range into just two bins, covering early and late phases (with a significance criterion of P = 0.025), or compare the iPRCs averaged over all phases (significance criterion of P = 0.05). For basic hypothesis testing (testing whether population means were different or whether a population mean was different from zero), either t-tests or the non-parametric Wilcoxon signed-rank test was used, depending on whether the data in question passed the Shapiro-Wilk normality test. Linear regression and analysis of variance (ANOVA) were also used where appropriate.

To determine whether differences between the iPRC measured with current injection and that obtained with synaptic stimulation could be due to the waveform of the synaptic current, we deconvolved the synaptic current waveform from the current iPRC, and we convolved the current pulse iPRC with the synaptic waveform (see Fig. 6D). These operations were performed using Mathematica’s ListConvolve and ListDeconvolve functions.

Measurements are means ± SD unless otherwise noted.

RESULTS

Infinitesimal phase response curves measured with EPSPs. In a phase model, the state of an intrinsically oscillating neuron is represented by the cell’s current position within its oscillatory cycle. That position, the phase, is sometimes expressed in radians, ranging from 0 to 2π (Galán 2009), but we express it as the fraction of the total oscillation period that has passed, ranging from 0 to 1 (Rinzel and Ermentrout 1998). If the cell’s intrinsic oscillation period is T, then the phase φ is defined as t/T. These states lie on a circle so that the extreme values of φ, 0 and 1, represent the same state (the spike), just as 0 and 2π represent the same angle measured in radians. The cell’s unperturbed oscillatory behavior is then represented by a steady increase in φ occurring as a...
ring at a rate \( k = 1/T \). Extrinsically applied current alters the rate of phase change in proportion to the strength of the current and as a function of the phase at which it is applied; that function is the iPRC, \( z(\phi) \). The differential equation describing the behavior of a phase model during application of a stimulus current \( I_{\text{stim}} \) is thus given by

\[
\frac{d\phi}{dt} = k + I_{\text{stim}} z(\phi).
\]

We seek to estimate \( z(\phi) \) from the response of the cell to synaptic input or injected current. However, we cannot directly measure the rate of change of \( \phi \) during stimulation; all we can do is measure the total change in phase, \( \Delta\phi \), caused by the stimulus during the ISI. If the stimulus is brief and small, so that \( z(\phi) \) is approximately constant during the stimulus, and the stimulus delivers a total charge \( q \) to the cell, then \( \Delta\phi \approx qz(\phi) \). This approximation becomes less accurate as either the stimulus duration or total charge increases, because the stimulus acts on an extended region of the iPRC rather than at one specific phase \( \phi \). Errors caused by stimulus duration can be fixed by deconvolution (Netoff et al. 2005a; Preyer and Butera 2005) if the stimulus amplitude is small enough, since the measured PRC can be viewed as convolution of the stimulus waveform with the iPRC. However, large stimuli, no matter how brief, can introduce errors even if Eq. 1 remains perfectly valid. This is because the stimulus itself can cause the cell to sweep through a range of phases over which \( z(\phi) \) is not constant. Given the brevity of our stimuli (see below), stimulus amplitude is a much more important source of error than stimulus duration, and it cannot be fixed with simple deconvolution. Nevertheless, given the size of the stimuli we used and the shape of STN PRCs, the distortions in our estimates of \( z(\phi) \) caused by inaccuracies of this approximation were small (this point is illustrated in Fig. 6A of our companion paper, Farries and Wilson 2012). Using the approximation \( \Delta\phi \approx qz(\phi) \), we can obtain the iPRC at \( \phi \) by dividing the stimulus-generated phase shift \( \Delta\phi \) by the stimulus charge \( q \). Equivalently, we can divide \( \Delta\phi \) by the membrane potential change \( \Delta V \) caused by the stimulus, since \( q = \text{constant}\) by the membrane potential change \( \Delta V \) caused by the stimulus, since \( q = \text{constant}\) by the cell capacitance. Because the charge delivered by synaptic input is not directly known, we find it more convenient to express measured iPRCs in units of \( \Delta\phi \) per millivolt of \( \Delta V \). Of course, such iPRCs can be converted into the more traditional units of \( \Delta\phi \) per unit charge by dividing by the capacitance.

The method for measuring the PRC using glutamatergic synaptic input, activated by stimulation of the internal capsule in the presence of GABA receptor antagonists, is illustrated in Fig. 1 for an example cell. Figure 1A shows the regular autonomous firing pattern of this cell, characteristic of STN cells in brain slices. Measurement of the raw PRC, the total phase shift \( \Delta\phi \) caused by the stimulus as function of the phase at which it was delivered (input phase), is illustrated in Fig. 1B. The input time of an EPSP is defined at the interval from the preceding spike to the point where the EPSP has reached 50% of its final amplitude. The “base ISI” is our estimate of what the ISI would have been without the stimulus; the input phase is the input time divided by the base ISI. The base ISI is, in turn, the average of spontaneous ISIs longer than the input time. The phase shift is the change in spike time caused by the stimulus, i.e., base ISI minus stimulated ISI, divided by the base ISI. Note that positive phase shifts correspond to shortening of the stimulated ISI. These measurements are made for at least 50 trials (usually 150 or more trials); the resulting raw PRC for our example cell is shown in Fig. 1C. There is a limit to the magnitude of positive phase shifts that can be observed during a single ISI, since the most the stimulus can do is trigger a spike immediately. The phase shift resulting from immediately triggering a spike is \( 1 - \phi \), where \( \phi \) is the input phase; the solid blue line in Fig. 1C marks this limit on positive phase shifts. Sometimes a spike is triggered during an EPSP; such trials are plotted as gray data points in Fig. 1C.

To convert the raw PRC into an estimate of the iPRC, we divide the stimulus-induced phase shifts by the average amount of depolarization caused by the EPSP. That depolarization is measured from the difference between the membrane potential at the end of the EPSP and the average voltage trajectory of spontaneous ISIs aligned to the time of the preceding spike (Fig. 1D); the end of the EPSP is determined by inspecting the time derivative of the EPSP (Fig. 1D, inset). On trials in which the entire EPSP falls within the stimulated ISI, dividing the phase shift by the EPSP amplitude gives the appropriately normalized phase shift per unit stimulus strength. However, this is not true of trials in which a spike is fired before the EPSP is complete; on these trials, the phase shift is divided only by the fraction of the EPSP amplitude that fell within the ISI (see MATERIALS AND METHODS), boosting the contribution of these trials to the iPRC. The normalized iPRC for our example cell, giving the phase shift per millivolt of membrane potential change as a function of input phase, is shown in Fig. 1E; the solid red line shows the smoothed iPRC. All subsequent analysis is performed on smoothed PRCs like this one. One factor that can be reasonably expected to affect the impact of a stimulus is spike threshold accommodation: in STN cells, larger EPSPs trigger a rapid but transient drop in spike threshold (Farries et al. 2010). This would lead one to expect EPSPs delivered at relatively late phases to be followed by spikes triggered at a lower threshold; this is certainly true of our example cell (Fig. 1F).

We measured iPRCs from the response to glutamatergic synaptic input in 89 STN cells, using EPSPs measuring 2.3 ± 1.1 mV (range 0.4–5.1 mV). The synaptic charge was delivered very quickly in most cases: the 10–90% EPSP rise time was 3.9 ± 1.9 ms (range 0.7–8.7 ms), and the 25–75% rise time was just 2.1 ± 1.2 ms (range 0.4–5.0 ms). The autonomous activity of these cells was fairly regular, with ISI coefficients of variation of 0.09 ± 0.04 (range 0.04–0.31). Figure 2A shows a summary of our results. The thin gray lines are individual iPRCs, the thick black line is the median iPRC, and the thinner black lines mark the 25th and 75th percentiles of the iPRC range (the quartile lines are shorter than most individual iPRCs because these population measures only cover the phase range (the quartile lines are shorter than most individual iPRCs because these population measures only cover the phase range covered by all individual iPRCs). On average, synaptic iPRCs start low at early phases, peak at middle phases, and decline again at later phases, although there is a great deal of variation from cell to cell. There is also a tendency for some iPRCs to have a second peak at very late phases (0.8–0.9); this can be seen in some individual iPRCs and is evident in the upper quartile line (Fig. 2A). This tendency toward a late peak in the iPRC is due almost entirely to trials in which a spike was triggered during the EPSP; if these trials are omitted, this late peak is abolished in almost all iPRCs (Fig. 2B). This late peak
could result from the amplifying influence of spike threshold accommodation, which tends to be most pronounced in trials where a spike was fired during the EPSP (e.g., Fig. 1F). However, the relationship between iPRC magnitude (phase shift per mV of EPSP amplitude) and the threshold at which the spike was triggered (relative to the threshold of spontaneous spikes) is extremely weak (linear regression fit slope \(-0.004, r^2 = 0.003, P = 0.003\)). This is not too surprising given that the EPSPs used to measure iPRCs here are generally smaller than those that evoke large and robust threshold drops in STN cells (Farries et al. 2010), but it casts some doubt on the notion that threshold accommodation could explain the late peak found in a subset of iPRCs. Some other aspect of the spike generation mechanism may alter the sensitivity of the cell to synaptic stimuli just prior to an action potential.

**Effect of EPSP amplitude and intrinsic firing rate.** The iPRC is an approximate description of an autonomously oscillating neuron’s response to input and should be most accurate for transient inputs of small magnitude. As the strength of the input is increased, there should come a point where the response of the neuron deviates from the response predicted by the iPRC; if that is the case, iPRCs estimated using inputs of varying strength should differ systematically from one another, at least for sufficiently powerful inputs. We examined this issue in our own data set, which covers a fairly wide range of EPSP sizes (Fig. 3A). We took two complementary approaches to assessing the effect of EPSP size. First, we divided our data into four groups based on EPSP size (Fig. 3A) and compared the average iPRCs of each group using ANOVA. The ANOVA makes no assumption about the form of the relationship between EPSP size and the iPRC, but it does require discrete groups defined by binning a continuous variable. In our second approach, we performed a linear regression of phase shift per millivolt against EPSP size for different input phases. Linear regression avoids binning the data by EPSP size but may fail to detect a nonlinear relationship between EPSP size and the
iPRC. Together, these two methods allow us to draw robust conclusions about the effect of EPSP size on the iPRC.

Average iPRCs grouped by EPSP size are very similar at early input phases but appear to diverge at later input phases (Fig. 3B). Specifically, larger EPSPs cause smaller phase shifts per unit stimulus, suggesting that EPSP-driven phase advances add sublinearly at late input phases. A one-way ANOVA revealed that the differences between the groups are not significant at most input phases, but the P value for this ANOVA drops below the $P = 0.05$ level at input phases $>0.84$ (Fig. 3C). At the latest phases ($\phi > 0.9$), this difference is highly significant ($P < 0.001$), surviving even the excessively stringent Bonferroni correction for multiple comparisons. Similarly, linear regression of phase shift per millivolt against EPSP size found no significant relationship between these factors at early input phases (Fig. 3D), but the regression slope first becomes significantly different from zero at the $P = 0.05$ level at $\phi = 0.68$ and is highly significant at later input phases ($P < 0.001$, $\phi > 0.85$). Both methods agree that there is no effect of EPSP size on iPRCs at $\phi < 0.6$ (Fig. 3E) and that there is one at $\phi > 0.85$ (Fig. 3F).

The STN cells in our sample exhibited a wide range of autonomous firing rates (Fig. 4A). Differences in intrinsic firing rate can be reasonably expected to result in differences in iPRCs, if only because differences in intrinsic rate are presumably due to differences in intrinsic conductances that could shape the iPRC. To assess the effect of intrinsic firing rate on the iPRC, we performed the same kind of analyses used to assess the effect of EPSP amplitude. When our cells are divided into four groups based on firing rate (Fig. 4A), the average iPRCs appear similar at early input phases, but the two groups with higher firing rates appear to produce more normalized phase shift at later input phases (Fig. 4B). When the differences between groups were compared by ANOVA at varying input phase, we found that the differences are not significant at early phases, first reach the $P = 0.05$ significance criterion at $\phi = 0.63$, and are highly significant ($P < 0.001$) at $\phi > 0.83$ (Fig. 4C). When we performed a linear regression of normalized phase shift against firing rate, we found that the slope of this relationship is significant at the $P < 0.05$ level for $\phi = 0.61$ and remain below the Bonferroni-corrected criterion ($P = 0.0012$) for $\phi > 0.67$ (Fig. 4D). As expected from these results, there is no effect of firing rate on normalized phase shift averaged over the first half of the ISI (Fig. 4E), but there is an effect on normalized phase shift averaged over later input phases (Fig. 4F). This effect, although highly significant, is quite weak (Fig. 4F), like the effect of EPSP amplitude.

Higher-order synaptic phase response curves. For some oscillatory systems, it is possible for a transient input to exert an effect that outlasts the oscillation cycle in which the input was delivered; such effects are not captured by simple phase models. Enduring perturbations of oscillatory behavior by a transient input can be represented by higher-order PRCs, which give fractional changes in oscillation period of successive cycles as a function of input phase of the cycle that received the stimulus (Oprisan et al. 2004). For example, the second-order PRC of an STN cell receiving an EPSP gives the fractional change in the ISI immediately following the stimulated ISI as a function of the input phase in the stimulated ISI. We examined higher-order PRCs of STN cells to determine whether EPSPs cause lasting effects on ISI duration that are inconsistent with the behavior of simple phase models. As with first-order iPRCs, we normalized higher order phase shifts by the strength of the stimulus, giving phase shift per millivolt of stimulus-induced membrane potential change. To ensure that second-order iPRCs did not include direct stimulus effects, we excluded trials in which the second spike of the stimulated ISI occurred during the EPSP, since the EPSP might persist into the next ISI on such trials. Figure 5A shows the median second-order PRC (bold line) along with the 25th and 75th percentiles (thin lines). Late input phases ($\phi > 0.7$) are not represented because of the exclusion of trials in which the EPSP might extend into the next ISI. The first ISI following a stimulus is significantly shorter (i.e., exhibiting positive phase shifts; Wilcoxon signed-rank test, $P < 0.0001$), although this effect is about an order of magnitude weaker than the changes associated with the first-order iPRC. The shortening of the first poststimulus ISI tends to be slightly stronger at later input phases (Fig. 5A).

We examined the effect of EPSP size and intrinsic firing rate on second-order PRCs. Figure 5B shows average second-order PRCs grouped by EPSP amplitude (same groups as in Fig. 3). There is a weak effect of EPSP size, in that the smallest EPSPs (<1.5 mV) do not exhibit significant second-order phase shifts when averaged over all input phases, a point we emphasize by showing the standard error of the mean for the smallest EPSP (Fig. 3B, thin black lines), whereas the other groups do (Wil-
Fig. 3. Effect of EPSP amplitude on synaptic iPRCs. A: histogram of EPSP amplitudes in our data set. The colored bars shown below the histogram mark the range of EPSP sizes included in the 4 groups into which we divided our data to investigate the effect of EPSP size. B: average iPRCs estimated from EPSPs of 4 different amplitude ranges. C: probability of type I error when testing the null hypothesis that the means in these 4 groups are all equal by 1-way analysis of variance (ANOVA). This analysis is repeated over narrow ranges of input phases (each 0.02 phase units wide), and the probability of mistakenly rejecting the null hypothesis that the means are equal in each narrow phase range is plotted (the abscissa for each point is the midpoint of its phase range). The red line shows the $P = 0.05$ criterion for rejecting the null hypothesis. D: probability of type I error when testing the null hypothesis that the regression slope of the relationship between normalized phase shift and EPSP size is equal to zero. As in C, this analysis is repeated on data taken from narrow phase windows (0.02 units wide) that collectively cover the ISI; the red line again shows the $P = 0.05$ criterion. E: relationship between normalized phase shift and EPSP size at relatively early phases. Each point represents data from 1 cell; the ordinate is the normalized phase shift of that cell’s iPRC averaged over input phases <0.6, whereas the abscissa is the size of the EPSP used to estimate that iPRC. The regression line for the relationship between these factors is shown in red. F: same as E, but using normalized phase shifts averaged over late input phases ($\phi > 0.85$).

coxon signed-rank test, $P < 0.02$). However, when a linear regression was performed on average normalized phase shift against EPSP size, no significant relationship was found. Figure 5C shows average second-order PRCs grouped by intrinsic firing rate (same groups as in Fig. 4). The main apparent effect is that second-order normalized phase shifts seem to depend on input phase at the lowest firing rates (<6 Hz) but not for higher firing rates. To test this observation statistically, we divided the input phase into quadrants ($\phi < 0.25, 0.25 \leq \phi < 0.5$, etc.) and performed an ANOVA to determine whether the mean normalized phase shift differed among quadrants. We found no statistically significant differences across quadrants for any of the firing rate groups, nor did we find statistically significant differences between groups at any input phase using the analysis methods that were applied to first-order iPRCs. Neither EPSP size nor intrinsic firing rate exerted a strong effect on second-order iPRCs.

Whatever weak phase dependence there is in second-order iPRCs disappears completely in third- and higher-order iPRCs, which show an overall shortening of the ISI that gradually dissipated as successive poststimulus ISIs are examined (not shown). This suggests that it may be more fruitful to examine the evolution of the normalized phase shift as a function time following the stimulus, regardless of the input phase or even which poststimulus ISI it is. We took all poststimulus ISIs from all cells and defined their time of occurrence as the time of the first spike bounding each ISI. We divided these ISIs into 1-ms bins based on time of occurrence relative to the stimulus and averaged the normalized phase shifts associated with each ISI within a bin. Note that this group ISIs by when they occur, not by their ordinal position in the sequence of poststimulus ISIs (e.g., the 50-ms bin may contain the first poststimulus ISI of a slow-firing cell and the third ISI of a rapidly firing cell). The result of this analysis is shown in Fig. 5D, revealing gradually declining phase advances that decay with a time constant of 72 ms. This suggests that most higher order effects in these experiments may simply result from a small but slowly decaying component of the EPSP. Even the weak phase dependence of the second-order iPRC can be explained in this way: the slow component of EPSP arriving at early phases would have decayed more than those arriving at later phases by the time the first poststimulus ISI began.

**Infinitesimal phase response curves measured with current pulses.** A common alternative strategy for measuring a cell’s iPRC is to use somatically injected current as the stimulus. The main advantage of this approach over synaptic iPRCs is the increased control over the stimulus: its amplitude and time course are under full experimental control, and any trial-to-trial variability in the stimulus is eliminated. We used 1- to 5-ms current pulses (comparable to the duration of our synaptic currents) of $\pm 20–100$ pA to remeasure iPRCs in the majority of cells in which we had measured synaptic iPRCs (64 of 89 cells); we also measured current pulse iPRCs in 2 additional cells that had not be studied synthetically. There was no effect of pulse duration on the shape of the measured PRC. Figure 6 shows how current pulse iPRCs were measured in an example cell (the same cell as shown in Fig. 1). We used both positive and negative current pulses to assess the iPRC in this cell, as we did in almost all cells (63 of 66 cells). The current pulses in this case were 2 ms in duration and $\pm 75$ pA in amplitude, our most common choice (27 of 66 cells). Figure 6A shows the raw PRCs, giving the total phase shift caused by the
stimuli, for both +75 pA (red) and −75 pA (blue) current pulses. Sometimes a spike was fired during a current pulse (almost always during depolarizing current pulses); such trials are plotted as gray data points.

With current pulse stimuli, we knew how much charge was delivered to the cell, so we could have normalized phase shifts with the charge supplied by the current pulse and obtained an estimate of the iPRC in its natural units. However, we wanted to compare estimates derived from synaptic and current pulse data quantitatively, so we continued to normalize PRCs by the membrane potential change caused by the stimulus. The average membrane potential trajectory following current pulses in this example cell is shown in Fig. 6B. About one-half of the initial membrane potential change decayed within a few milliseconds, after which the membrane potential appears to have resumed its prestimulus rate of rise. This rapid partial decay probably reflects the redistribution of injected charge: initially, that charge is concentrated near the site of injection, and some time is required for it to become distributed more evenly across the cell’s membrane. To account for this effect, we waited 5 ms after the termination of a current pulse before measuring the change it caused in the membrane potential. The measured potential changes caused by positive and negative pulses of equal magnitude were always close but rarely identical, and this difference was significant (paired t-test, \( p = 0.003 \)). Nevertheless, we did not normalize the phase shifts of equal but opposite current pulses with different \( \Delta V \) magnitudes; if the difference in \( \Delta V \) affects the ability of equal-magnitude pulses to alter spike times, that should be reflected in their iPRCs rather than normalized away. Thus phase shifts were normalized by the average \( \Delta V \) measured for positive and negative current pulses (with the appropriate sign given the polarity of the pulse). Figure 6C shows normalized current pulse iPRCs for our example cell. The solid blue and red traces show smoothed iPRCs for +75-pA current pulses, respectively. These iPRCs were very similar except at late input phases (\( \phi > 0.85 \)); the black trace shows the smoothed iPRC of the two pulses combined. Figure 6D plots this smoothed combined current pulse iPRC with this cell’s synaptic iPRC.

As Fig. 6D shows, the current pulse and synaptic iPRCs for this example cell were quite different at early input phases. To determine the extent to which this difference could be due to differences in the time course of the stimulus currents, we obtained a second estimate of the synaptic iPRC by deconvolving the raw (unnormalized) synaptic PRC with the synaptic current waveform (Fig. 6D, thin blue line); the synaptic current was calculated from the time derivative of the EPSP waveform. The deconvolved iPRC was noisy near the endpoints (\( \phi \sim 0/1 \); not shown) but was otherwise essentially indistinguishable from the iPRC obtained simply from dividing the raw phase shift by the EPSP size (Fig. 6D, thick green line). Conversely, we convolved the current pulse iPRC with the synaptic current waveform (normalized to an integral of 1) to see the effect of the synaptic waveform on the apparent shape of the iPRC (Fig. 6D, thin red line). Again, the shape and duration of the synaptic waveform had virtually no effect on the iPRC. This illustrates why we did not use deconvolution in our iPRC measurements:

\[ \text{Phaseshift per mV} = \frac{\text{phase shift}}{\text{input phase}} \]

\[ \text{Phase shift per mV} = \frac{\Delta V}{\Delta \phi} \]

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both the synaptic and injected currents were far too brief (<5% of the typical oscillation period) to have a significant effect on iPRC shape.

Figure 7A shows the individual current pulse iPRCs we measured in 66 cells (thin gray lines), using combined data from both positive and negative current pulses where available, plotted with the median current pulse iPRC (thick black line) and 25/75th percentiles of that population (thin black lines). On average, current pulse iPRCs rose through most of the ISI and achieved a maximum somewhere between input phases of 0.6 and 0.8 before declining at later input phases. When iPRCs measured with positive and negative current pulses were compared (Fig. 7B), we found that they were very similar at early input phases but diverged at later phases; this difference was significant for \( \phi > 0.75 \) (\( P < 0.001 \)). Figure 7C shows average second-order current pulse iPRCs. Higher-order iPRCs measured with negative pulses were never significantly different from zero at any input phase or for any order (Wilcoxon signed-rank test). The same is true of positive current pulses for third- and higher-order iPRCs, but for second-order iPRCs, positive current pulses showed significant phase delays for input phases above 0.79. In other words, the first poststimulus ISI following a positive current pulse tended to be slightly longer than the average spontaneous ISI if that positive current pulse was delivered in the final fifth of the preceding ISI.

The difference between first-order iPRCs measured with positive and negative current pulses can be explained by their divergent effects on spike threshold (Fig. 7D). Negative current pulses produce a very small (<0.1 mV) but statistically significant drop in the threshold of the next spike if delivered at \( \phi < 0.75 \) (Wilcoxon signed-rank test, \( P < 0.001 \)). Positive current pulses, on the other hand, produce a very small increase in spike threshold at early phases but produce a much more substantial threshold drop at late input phases (\( \phi > 0.8 \)). This difference in threshold behavior would be expected to boost the impact of positive current pulses relative to negative current pulses at late input phases. If that is indeed the case, there should be a relationship between spike threshold and normalized phase shift for positive current pulses delivered at late input phases. There is in fact such a relationship: if we examine phase shifts at \( \phi > 0.8 \), we find that cells in which the stimulus evoked spikes at lower thresholds (relative to their spontaneous spike threshold) tended to show larger normalized phase shifts (Fig. 7E). The difference between second-order iPRCs measured with positive and negative current pulses might also be explained by threshold effects. Depolarizing stimuli that transiently lower spike threshold also tend to evoke smaller but much longer lasting rises in spike threshold after the threshold drop is over (Farries et al. 2010). This effect probably explains why depolarizing current pulses evoked relatively large threshold drops at late input phases while producing small rises in threshold at early phases (Fig. 7D). This suggests that late-phase depolarizing pulses that lower the threshold of the first poststimulus spike should raise the threshold of the second poststimulus spike, and in fact they do (not shown). That could explain why late-phase depolarizing pulses lengthen the next ISI (Fig. 7C). However, we found no significant correlation between the rise in threshold of the second poststimulus spike and the normalized phase shift of the second-order iPRC.

One of our primary objectives was to quantitatively compare iPRCs measured with synaptic input to those measured with somatically injected current, to determine how well such iPRCs can predict a cell’s response to synaptic input. The average synaptic and current pulse iPRCs are plotted together in Fig. 7F. In evaluating this graph, it is important to recall that in the majority of cases, synaptic and current pulse iPRCs were
Different input phases: the average synaptic iPRC achieved a significant at the kind of stimuli very different shapes. This difference was phase early in the ISI but were equivalent to current pulses at were more than twice as effective as current pulses at altering EPSPs might have a small but very long-lasting component. This fact to emerge from this comparison is that EPSPs could account for both the (apparent) additional effectiveness of EPSPs over injection current and for the difference in overall iPRC shape. The leading candidate to mediate a slow EPSP component is the NMDAR; EPSPs evoked in the STN by internal capsule stimulation are known to include an NMDAR component, and this component has decay kinetics that are broadly consistent with what we have observed in poststimulus ISIs (Farries et al. 2010). Furthermore, we obtained preliminary data suggesting that the difference between synaptic and current pulse iPRCs was smaller in cells whose slices had been incubated in the NMDAR antagonist MK-801 (5 cells; not included in the present study). In view of these factors, we chose to examine the effect of the NMDAR antagonist APV on iPRCs, with special attention to the difference between synaptic and current pulse iPRCs.

We were able to measure synaptic iPRCs before and after bath application of 50 μM APV in 14 cells. APV had no significant effect on synaptic iPRCs at any input phase (Fig. 8A), nor was there a significant difference in normalized phase shift averaged over all input phases (paired t-tests). This is not to say measured in the same cells, and the results are virtually unchanged if we limit ourselves to the 64 cells that were assessed with both synaptic and current pulse stimuli. The most striking fact to emerge from this comparison is that EPSPs were more than twice as effective as current pulses at altering phase early in the ISI but were equivalent to current pulses at very late input phases, giving iPRCs measured using the two kinds of stimuli very different shapes. This difference was significant at the $P = 0.05$ level for all $\phi < 0.72$ and was significant at the Bonferroni-corrected criterion ($P = 0.0012$) for all $\phi < 0.68$. On the other hand, this difference was not significant at the $P = 0.05$ level for any $\phi > 0.72$. The average synaptic and current pulse iPRCs reached their peaks at very different input phases: the average synaptic iPRC achieved a maximum at $\phi = 0.43$, whereas the average current pulse iPRC peaked much later, at $\phi = 0.77$. This means that the difference between these iPRCs cannot be explained by a simple measurement error in the amount of potential change produced by the stimuli: no amount of rescaling can make these two average iPRCs correspond at all input phases.

Effect of APV on infinitesimal phase response curves. Our analysis of higher-order synaptic iPRCs suggested that evoked EPSPs might have a small but very long-lasting component (Fig. 5). Obviously, this component is not accounted for by our measurement of EPSP amplitude, since those measurements are made only 3–15 ms after EPSP onset. Thus the difference between synaptic and current pulse iPRCs may be explained by the fact that EPSPs are really larger than our measurements indicate; when appropriately normalized, the difference between synaptic and current pulse iPRCs may be reduced or even disappear. The effect of this hypothetical slow EPSP component should be phase dependent, since EPSPs initiated at early phases will have more time to deliver additional charge via the slow component, whereas the charge delivered by late-phase EPSPs will be largely limited to what is accounted for by our amplitude measurements. In this way, a slow EPSP component could account for both the (apparent) additional effectiveness of EPSPs over injection current and for the difference in overall iPRC shape. The leading candidate to mediate a slow EPSP component is the NMDAR; EPSPs evoked in the STN by internal capsule stimulation are known to include an NMDAR component, and this component has decay kinetics that are broadly consistent with what we have observed in poststimulus ISIs (Farries et al. 2010). Furthermore, we obtained preliminary data suggesting that the difference between synaptic and current pulse iPRCs was smaller in cells whose slices had been incubated in the NMDAR antagonist MK-801 (5 cells; not included in the present study). In view of these factors, we chose to examine the effect of the NMDAR antagonist APV on iPRCs, with special attention to the difference between synaptic and current pulse iPRCs.

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that APV had no effect on glutamatergic synaptic transmission in these experiments: APV did reduce the total amount of phase shift evoked by EPSPs (Fig. 8B). Specifically, the difference in raw PRCs was significant at the \( P = 0.05 \) level for almost all \( \phi < 0.8 \) (although it never achieved the Bonferroni-corrected criterion of \( P < 0.0012 \)), and there was a significant difference in total phase shift averaged over all input phases (paired \( t \)-test, \( P = 0.02 \)). Evidently, the effect of APV on first-order PRCs is mainly due to an effect on the size of fast EPSP rather than on some slow EPSP component that is not captured by our measurements. The average EPSP amplitude was 12% smaller after APV application, although this difference was not significant (paired \( t \)-test, \( P = 0.11 \)). We measured current pulse iPRCs under control conditions in all 14 cells and obtained current pulse iPRCs in APV in 9 of these cells. Not surprisingly, APV had no effect on the cell’s response to current pulses (Fig. 8C). In a final effort to detect any contribution of NMDARs to the difference between synaptic and current pulse iPRCs, we examined the pairwise difference between these curves under control conditions and after application of APV (Fig. 8D). We found no significant change in the pairwise difference between synaptic and current pulse iPRCs upon application of APV, and the pairwise difference averaged over \( \phi < 0.6 \) was significantly different from zero in both control (\( t \)-test, \( P \ll 0.001 \)) and APV (\( t \)-test, \( P = 0.007 \)).

The failure of NMDAR blockade to affect the gap between synaptic and current pulse iPRCs suggests that the hypothetical slow EPSP might be mediated by something else, in which case the persistent shortening of ISIs seen in higher order synaptic iPRCs would also be insensitive to APV. However, that was not the case. When averaged over all input phases, the

second-order normalized phase shift was significantly different from zero in control (\( P = 0.001 \)) but not after application of APV (\( P = 0.09 \), Wilcoxon signed-rank test); furthermore, control and APV values were significantly different from each other (paired \( t \)-test, \( P = 0.01 \)). Looking at the average second-order iPRCs (Fig. 8E), it appears that the effect of APV depends on input phase, reducing or abolishing the phase shift at late input phases while leaving the shift at early input phases untouched. Statistical analysis only partially supports this impression. Second-order iPRCs in control and APV were significantly different from each other at late input phases (\( \phi > 0.8 \); \( P = 0.02 \), paired \( t \)-test) but not at early input phases (\( \phi < 0.5 \); \( P = 0.59 \), paired \( t \)-test). On the other hand, second-order iPRCs measured in APV were not significantly different from zero at early or late phases (\( P = 0.23 \) or \( P = 0.57 \); Wilcoxon signed-rank tests), whereas in control they were significantly different at both early and late phases (\( P = 0.002 \) and \( P = 0.0004 \); \( t \)-tests). Figure 8F plots average normalized phase shift as a function of time since the stimulus (similar to Fig. 5D) in control and after APV application. APV almost entirely abolished the slowly decaying excitatory effect seen in control: average normalized phase shift in the first 50 ms after stimulation changed from 0.0070 in control to 0.0017 in APV, and this difference was significant (\( t \)-test, \( P < 0.0001 \)). In fact, the normalized phase shift in this time window in APV was not significantly different from zero (\( t \)-test, \( P = 0.09 \)).

**Diversity of infinitesimal phase response curves.** Most of the median and average iPRCs we have shown are fairly bland, exhibiting relatively little phase dependence in the case of synaptic iPRCs or a simple monotonic rise over most input phases in the case of current pulse PRCs (Fig. 7F). It is not
immediately obvious whether these average iPRCs should be regarded as the best representatives of the true subthalamic iPRCs or whether they conceal functionally important cell-to-cell variation in iPRC structure. Figures 2A and 7A clearly show a great deal of variability in individual iPRCs, but at least some of this is due to measurement error. This is especially true of iPRCs estimated with weak inputs, since noise from natural ISI variability is amplified by normalization to a small stimulus amplitude. We do not have a foolproof method for distinguishing real iPRC variability from measurement error, but in cases where the iPRC was measured twice (by EPSP and by current injection), comparing the two iPRCs could help address this question despite the systematic difference between iPRCs measured using these two methods.

Figure 9 shows iPRCs measured in a sample of 12 STN cells, with synaptic (green) and current pulse (black) iPRCs plotted together for each cell. When these cells are classified by the structure of their synaptic iPRCs, some appear to be relatively insensitive to input phase (Fig. 9, first row). The current pulse iPRCs of these cells seem to follow that pattern as well, save for a tendency to be more sensitive to inputs arriving at late phases. Overall, these cells roughly conform to the pattern observed in average iPRCs. Other cells have synaptic iPRCs that start low and grow by at least a factor of 4 at later input phases (Fig. 9, third row). The first two examples show peak input sensitivity near $\phi = 0.5$, followed by decline; they resemble the general pattern of phase dependence seen in the average synaptic iPRC, but with a much greater level of variation by input phase. The last example in this row is interesting because it shows a very sharp transition from low to high input sensitivity around $\phi = 0.2$ in both synaptic and current pulse iPRCs. Finally, and most controversially, we sometimes see evidence of more complex patterns in iPRCs (Fig. 9, fourth row). The left panel of this row shows a cell that appears to have two distinct peaks in its iPRCs, one at $\phi \approx 0.25$ and one at $\phi \approx 0.75$. The other two panels show iPRCs with a small dip at early phases ($\phi \approx 0.3$); these would certainly be ascribed to measurement noise were it not for the fact the dip is shared across synaptic and current pulse iPRCs (but perhaps is still merely coincidental). We do not think these four groups represent distinct classes of iPRC, but taken as a whole they do suggest that there is real and substantial variation in iPRC structure across STN cells and that the average iPRC is not necessarily a good representative of the iPRC of most STN cells.

**Synaptic currents underlying the response to stimulation.** As we have indicated, one strategy for devising and using phase models entails measuring the iPRC using somatic current injection (e.g., Galán et al. 2005; Phoka et al. 2010; Reyes and Fetz 1993; Tateno and Robinson 2007) and combining that with some estimate of the synaptic current derived from voltage-clamp data. One method, dynamic clamp of a simulated synaptic conductance,
even uses the estimated synaptic current, injected somatically, to measure the PRC (Netoff et al. 2005a, 2005b; Oprisan et al. 2004; Preyer and Butera 2005; Sieling et al. 2009). The difference we observed between synaptic and current pulse iPRCs suggests that this will not be an effective strategy in subthalamic neurons, but it is still instructive to compare the synaptic currents inferred from current-clamp recordings with the synaptic currents recorded in voltage clamp. We recorded the synaptic currents underlying a subset of our synaptic iPRCs in voltage clamp (47 of 89 cells) at holding potentials of 67–110 mV (after correction for postrecording voltage offset and series resistance error). Because we used the perforated-patch technique, the series resistance was highly variable (39 ± 17 MΩ, range 16–75 MΩ, but in some instances it was fairly low (20 cells with series resistance <30 MΩ).

The time derivative of the EPSP is proportional to the synaptic current delivered to the perisomatic region accessed by our recording electrode. Given our past observation that subthalamic neurons exhibit a near-zero slope conductance over a wide range of subthreshold membrane potentials (Farries et al. 2010), one might expect the time derivative of EPSPs to closely resemble excitatory postsynaptic currents (EPSCs) recorded in voltage clamp. They do not: the derivative of the EPSP peaks earlier and decays faster than the EPSC, even in recordings with relatively low series resistance (Fig. 10A shows an example with a series resistance of 26 MΩ). In fact, we saw no significant relationship between series resistance and the EPSC decay time constant, although there was a slight trend toward longer time constants at higher series resistances (fit slope = 0.08, P = 0.052, r² = 0.08). Figure 10B shows the distributions of decay time constants of EPSP derivatives (black bars) and of EPSCs (red bars). In recordings featuring both current-clamp and voltage-clamp measurements (n = 47),
the decay time constant of the EPSP derivative (1.2 ± 0.7 ms, range 0.3–3.5 ms) was in every case shorter than the EPSP decay time constant measured in the same cell (7.3 ± 4.9 ms, range 1.1–30.8 ms). This difference was highly significant (P < 0.000001, paired Wilcoxon signed-rank test). Although the time course of synaptic current inferred from current-clamp data was very different from that of EPSCs measured in voltage clamp, there was at least a strong correlation between the size of the EPSP and the integral of the EPSC (Fig. 10C).

Because EPSPs are generated by a conductance change rather than current injection, EPSP amplitude should depend on membrane potential, and one might expect dynamic clamp of a synaptic conductance to more accurately represent the effect of synaptic input than injection of a current waveform. However, when we examined the effect of membrane potential on EPSP size, we not only failed to observe an extrapolated reversal potential near 0 mV, as one would expect for glutamatergic EPSPs, we did not even see a consistent decline in EPSP amplitude with increasing depolarization. The slope of the relationship between EPSP size and membrane potential was negative in only 40 of 89 cells and was significantly less than zero (at the P < 0.05 level) in only 10 cells. Each cell’s EPSPs only sampled a limited range of potentials, but we can extend that range by combining data from many cells, with EPSP amplitude at a shared reference potential (±57 mV), and the red line is the linear fit to all the data. Gray data points are individual EPSPs, and the red line is the linear fit.

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The relationship between EPSP size and membrane potential in the combined data set (Fig. 10D) was significantly less than zero (P = 0.005), but the relationship was extremely weak (r² = 0.001, fit slope = -0.004, extrapolated reversal potential = 176 mV). In summary, the synaptic current arriving in the soma is reshaped by the dendrites and behaves more like an injected current than a conductance change (this is unlikely to be true of GABAergic inhibitory postsynaptic potentials, whose reversal potential lies in the subthreshold range). It has long been understood that somatic EPSPs caused by dendritic conductance changes will not be as sensitive to somatic membrane potential as a simple single-compartment model would predict (Rall 1967), but the extent of this divergence in subththalamic neurons may come as a surprise. Both the fast time course and lack of sensitivity to somatic membrane potential of synaptic current arriving at the soma may be due to the influence of voltage-dependent K+ conductances in the dendrites (Wilson 1995).

DISCUSSION

The purpose of using phase models to represent neurons is to obtain tractable models that still capture the behavior of real neurons with sufficient accuracy. Phase models cannot possibly reproduce the full repertoire of phenomena exhibited by neurons receiving arbitrary patterns of synaptic input. Nevertheless, our data indicate that the response of STN cells to excitatory synaptic input is reasonably well described by an iPRC. Specifically, the average iPRC changes relatively little as EPSP size is increased, and even the dependence on intrinsic firing rate is comparatively minor. The effects of EPSPs on poststimulus ISIs, effects that could not be accounted for within a simple phase model, appear to be minimal and might be explained by the action of a slow EPSP component that could be accommodated within a simple phase model. NMDA-type glutamate receptors, whose slow kinetics and voltage dependency might have introduced complications, appear sim-
were adjusted to give the same amount of depolarization as the measured with EPSPs and current pulses when current pulses motor cortex found no systematic difference between PRCs pulses. A study of PRCs in pyramidal neurons of cat sensori-
oscillations often appear in Parkinson’s disease (Bergman et al. 1994), but synchronous
Because STN cells are glutamatergic and all synaptic iPRCs
another neurotransmitter), but voltage-clamp analysis of the synaptic currents evoked in STN cells by stimulation of the internal capsule found only fast AMPAR-mediated and slower NMDAR-mediated components, once GABA receptors were blocked (Farries et al. 2010). Another possibility is that glutamatergic input triggers some process in the dendrites that gradually
If that is the case, then there would be little gradual addition of charge throughout the ISI; NMDAR current flowing during the AMPAR-mediated EPSC would increase the size of the fast EPSP, while charge passing through NMDARs during the action potential might shorten the next ISI without contributing to the first-order iPRC. Of course, it is possible, even likely, that NMDARs make a small additional contribution to first-order iPRCs that could be detected with enough statistical power. Even so, our results clearly demonstrate that NMDARs can never explain the large gap between synaptic and current pulse iPRCs.
If NMDARs cannot explain the difference between synaptic and current pulse iPRCs, what can? There could be a slow EPSP component that is mediated by another receptor type (possibly even another neurotransmitter), but voltage-clamp analysis of the synaptic currents evoked in STN cells by stimulation of the internal capsule found only fast AMPAR-mediated and slower NMDAR-mediated components, once GABA receptors were blocked (Farries et al. 2010). Another possibility is that glutamatergic input triggers some process in the dendrites that gradually delivers additional charge to the axosomatic region, but given the voltage-clamp data, it would have to be something disrupted either by voltage clamp or by the internal cesium used to improve space clamp in those experiments (Farries et al. 2010). Finally, it is possible that there is some flaw in the current pulse method for measuring iPRCs in STN cells and that synaptic stimuli give a better estimate of the cell’s true iPRC.
Another major finding was our observation that iPRCs vary considerably from cell to cell. Although all STN iPRCs are type I, they exhibit almost any variation permitted within that broad class. The structure of a cell’s iPRCs must depend on the intrinsic conductances it expresses, yet all STN cells appear to express the same set of conductances and fall within a single cell type (Bevan and Wilson 1999; Nakanishi et al. 1987). Clearly, there is some quantitative variability in the expression of these conductances that is manifest, for example, in variability of the intrinsic firing rate, but without a theory that explains how different intrinsic conductances affect the structure of the iPRC, it is difficult to know whether the variability in conductances one might reasonably find in STN cells is sufficient to explain the iPRC variability we observed. A theoretical understanding of STN iPRCs would ideally explain how the cell’s intrinsic conductances shape its
iPRC and could help identify which aspects of iPRC variability represent real differences (as opposed to mere measurement error). A theoretical approach might also help us address some of the other questions raised by our results, including the nature of the difference between synaptic and current pulse iPRCs and the reasons why the iPRC description works as well as it does in the STN. We develop a theoretical approach to understanding subthalamic iPRCs in a companion paper (Farries and Wilson 2012).

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DISCLOSURES
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AUTHOR CONTRIBUTIONS
M.A.F. and C.J.W. conception and design of research; M.A.F. performed experiments; M.A.F. analyzed data; M.A.F. interpreted results of experiments; M.A.F. prepared figures; M.A.F. drafted manuscript; M.A.F. and C.J.W. edited and revised manuscript; M.A.F. and C.J.W. approved final version of manuscript.

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