Phase Response Curves of Subthalamic Neurons Measured with Synaptic Input and Current Injection

Running Head: Phase Response Curves in the Subthalamic Nucleus

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ABSTRACT

Infinitesimal phase response curves (iPRCs) provide a simple description of the response of repetitively firing neurons, and may be used to predict responses to any pattern of synaptic input. Their simplicity makes them useful for understanding the dynamics of neurons, when certain conditions are met. For example, the sizes of evoked phase shifts should scale linearly with stimulus strength, and the form of the iPRC should remain relatively constant as firing rate varies. We measured the PRCs of rat subthalamic neurons in brain slices using corticosubthalamic EPSPs (mediated by both AMPA- and NMDA-type receptors) and injected current pulses, and used them to calculate the iPRC. These were relatively insensitive to both the size of the stimulus and to the cell’s firing rate, suggesting that the iPRC can predict the response of STN cells to extrinsic inputs. However, the iPRC calculated using EPSPs differed from that obtained using current pulses. EPSPs (normalized for charge) were much more effective at altering the phase of subthalamic neurons than current pulses. The difference was not attributable the extended time course of NMDAR-mediated currents, being unaffected by blockade of NMDARs. The iPRC provides a good description subthalamic neurons' response to input, but iPRCs are best estimated using synaptic inputs rather than somatic current injection.

INTRODUCTION

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, 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; b; Stiefel et al. 2008; Tateno and Robinson 2007; Tsubo et
al. 2007) or simulated conductance changes (Netoff et al. 2005a; Netoff et al. 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;
vан 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 timescales as the 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). 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 somatically injected current had the same effect on spike timing as synaptically
generated conductance changes, we compared iPRCs measured with EPSPs to those measured with 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 - 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, 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 corticosubthalamic 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, 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 40X 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 µg/mL; otherwise we used gramicidin at 0.5 - 1 µg/mL. Electrodes were tip-filled with gramicidin-free solution and back-filled 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 analog-to-digital converter (Instrutech Corporation, 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_A channel blocker picrotoxin (150 µM) and the GABA_B receptor antagonist CGP-55845 (1 µM). In addition, the NMDAR antagonist DL-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 define the time of a spike as the time during the
action potential rising phase at which the rate of rise is maximal. Spike threshold is
defined as the potential at which the second derivative of the voltage trajectory
approaching a spike reached 50 mV/ms². Phase response curves (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 are 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
depend 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 seconds 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. However, 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⁺ conductance and more conventional
positive slope conductances (Farries et al. 2010). Thus, by confining our measurements
to EPSPs arriving 20-50% though the autonomous ISI, we obtained synaptic potentials
that were largely confined to the zero-slope region of the STN cell’s IV 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 synaptically-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, inspection of the first and
second derivative waveforms 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 (final section of the Results), 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, 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 infinitesimal PRCs (iPRCs) were estimated by dividing the total phase shift by the EPSP amplitude to give the normalized phase shift per mV. 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
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 value 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 magnitude, but for current pulses the method for determining that fractional magnitude was much simpler: the timing of the spike relative to pulse onset determined the fraction of the pulse falling within the stimulated ISI, and the associated phase shift was normalized by that fraction times the total measured magnitude. We used several different current pulse sizes and durations to measure iPRCs. The most common pulse duration was 2 ms, 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 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 introduces a
problem with multiple comparisons—a comparison of two populations of iPRCs becomes
41 - 43 separate tests. We use 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 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). Basic hypothesis
testing (testing whether population means were different, or whether a population mean
was different from zero) used either t tests or the nonparametric Wilcoxon signed-rank
test, 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 (Fig. 6D). These operations were performed using Mathematica's ListConvolve and ListDeconvolve functions.

Measurements are reported as mean ± standard deviation 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 $2\pi$ (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 $T$, and $t$ is the time since the last spike (without any perturbing stimulus arriving in that interval), then the phase $\phi$ is defined as $t/T$. These states lie on a circle, so that the extreme values of $\phi$, 0 and 1, represent the same state (the spike), just as 0 and $2\pi$ represent the same angle measured in radians. The cell's unperturbed oscillatory behavior is then represented by a steady increase in $\phi$ occurring at a rate $k \equiv 1/T$. 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 infinitesimal PRC (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

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

(1)

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 interspike interval (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 \( \Delta \phi = q \cdot z(\phi) \). This approximation becomes less accurate as either the stimulus duration or total charge increase, because the stimulus acts on an extended region of the iPRC rather than at one specific phase \( \phi \). Errors caused 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 Equation 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 that 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
Using the approximation $\Delta \varphi = q z(\varphi)$, we can obtain the iPRC at $\varphi$ by dividing the stimulus-generated phase shift $\Delta \varphi$ by the stimulus charge $q$.

Equivalently, we can divide $\Delta \varphi$ by the membrane potential change $\Delta V$ caused by the stimulus, since $q$ is equal to $\Delta V$ times the cell capacitance. Because the charge delivered by synaptic input is not directly known, we found it more convenient to express measured iPRCs in units of $\Delta \varphi$ per millivolt of $\Delta V$. Of course, such iPRCs can be converted into the more traditional units of $\Delta \varphi$ 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 Figure 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 $\Delta \varphi$ caused by the stimulus as function of the phase at which it was delivered (input phase)—is illustrated in Figure 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 Figure 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 $1 - \varphi$, where $\varphi$ is the input phase; the solid blue line in Figure 1C marks this limit on positive phase shifts.

Sometimes, a spike is triggered during an EPSP; such trials are plotted as gray dots in Figure 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 Methods), boosting the contribution of these trials to the iPRC. The normalized iPRC for our example cell, giving the phase shift per mV 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 $2.3 \pm 1.1$ mV (range, 0.4 - 5.1 mV). The synaptic charge was delivered very quickly in most cases: the 10-90% EPSP rise time was $3.9 \pm 1.9$ ms (range, 0.7 - 8.7 ms) and the 25-75% rise time was just $2.1 \pm 1.2$ ms (range, 0.4 - 5.0 ms).

The autonomous activity of these cells was fairly regular, with ISI coefficients of variation of $0.09 \pm 0.04$ (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 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 towards 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 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 4 groups
based on EPSP size (Fig. 3A) and compared the average iPRCs of each group using
analysis of variance (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 mV 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 were 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 mV 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). In any case, the relationship between normalized phase shift and EPSP size is quite weak even at late input phases ($r^2 = 0.21$); overall the iPRC is remarkably insensitive to the size of the stimulus used to measure it.

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 4 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 $\varphi = 0.63$, and are highly significant ($p < 0.001$) at $\varphi > 0.83$ (Fig. 4C). When we performed a linear regression of normalized phase shift against firing rate, we found that the slope of this relationship was significant at the $p < 0.05$ level by $\varphi = 0.61$ and remained below the Bonferroni-corrected criterion ($p = 0.0012$) for $\varphi > 0.67$ (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, though 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 normalize higher-order phase shifts by the strength of the stimulus, giving phase shift per mV of stimulus-induced membrane potential change. To ensure that second-order iPRCs do not include direct stimulus effects, we exclude trials in which the second spike of the stimulated ISI occurred during the EPSP, since the EPSP may persist into the next ISI on such trials. Figure 5A shows the median second-order PRC (thick 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 (Wilcoxon signed-rank test, $p < 0.02$). However, when a linear regression is performed on average normalized phase shift against EPSP size, no significant relationship is 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 if 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 exert 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 dissipates as successive poststimulus ISIs are examined (not shown). This suggests that it might 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 groups 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 Figure 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 begins.

**Infinitesimal Phase Response CurvesMeasured 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 - 5 ms current pulses (comparable to the duration of our synaptic currents) of
±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 been studied synaptically. 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 ±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 circles.

With current pulse stimuli, we know how much charge was delivered to the cell,
so we could normalize phase shifts with the charge supplied by the current pulse and
obtain an estimate of the iPRC in its natural units. However, we want to compare estimates derived from synaptic and current pulse data quantitatively, so we continue 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 Figure 6B. About half of the initial membrane potential change decays within a few milliseconds, after which the membrane potential appears to resume 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 wait 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 are always close but rarely identical, and there is a systematic difference between them: the measured magnitude of $\Delta V$ for negative pulses is 3.4% smaller on average than for positive pulses, and this difference is significant (paired t test, $p = 0.003$). Nevertheless, we do 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 are 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 and +75 pA current pulses, respectively. These iPRCs are very similar save at late input phases ($\phi$...
As Figure 6D shows, the current pulse and synaptic iPRCs for this example cell are 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 is noisy near the endpoints (\( \phi \sim 0/1 \), not shown) but is 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: 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 rise through most of the ISI and achieve 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 are compared (Fig. 7B), we find that they are very similar at early
input phases but diverge at later phases; this difference is 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 show 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 a 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 evoke relatively large threshold drops at late input phases while producing small rises in threshold at early phases (Fig. 7D). That 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 is 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 Figure 7F. In evaluating this graph, it is important to recall that in the majority of cases synaptic and current pulse iPRCs were 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 are more than twice as effective as current pulses at altering phase early in the ISI but are equivalent to current pulses at very late input phases, giving iPRCs measured using the two kinds of stimuli very different shapes. This difference is significant at the $p = 0.05$ level for all $\phi < 0.72$.
and is significant at the Bonferroni-corrected criterion \((p = 0.0012)\) for all \(\phi < 0.68\). On the other hand, this difference is not significant at the \(p = 0.05\) level for any \(\phi > 0.72\).

The average synaptic and current pulse iPRCs reach their peaks at very different input phases: the average synaptic iPRC achieves a maximum at \(\phi = 0.43\), while the average current pulse iPRC peaks 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 may 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 it accounted for by our amplitude measurements. In this way, a slow EPSP component could account for both the (apparent) additional effectiveness of EPSPs over injected current and for the difference in overall iPRC shape. The leading candidate to mediate a
slow EPSP component is the NMDA-type glutamate receptor (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 is 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 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 ϕ < 0.8 (although it never achieved the Bonferroni-corrected criterion of p < 0.0012), and there is 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 is 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 has 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 $\phi < 0.6$ is significantly different from zero in both control (t test, $p << 0.001$) 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 is not the case. When averaged over all input phases, the second-order normalized phase shift is 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 are 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 are significantly different from each other at late input phases ($\phi > 0.5; p = 0.02$, 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 are not significantly different from zero at early or late phases ($p = 0.23$, $p = 0.57$; Wilcoxon signed-rank tests), whereas in control they are
at both early and late phases ($p = 0.002$, $p = 0.0004$; $t$ 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 abolishes the slowly-decaying excitatory effect seen in control: average normalized phase shift in the first 50 ms after stimulation goes from 0.0070 in control to 0.0017 in APV, and this difference is significant ($t$ test, $p < 0.0001$). In fact, the normalized phase shift in this time window in APV is not significantly different from zero ($t$ test, $p = 0.09$).

Diversity of Infinitesimal Phase Response Curves

The median and average iPRCs we have shown in most of the figures 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. Classifying these cells by the structure of their synaptic iPRCs, some appear to be relatively insensitive to input phase (Fig. 9, top 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 high at early input phases and decline as input phase increases (Fig. 9, second row). Their current pulse iPRCs do not really follow this pattern (with the exception of the middle cell), but they do deviate from the average current pulse iPRC by peaking at relatively early input phases ($\phi < 0.5$) and declining thereafter. A third group of 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 on 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, bottom 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 4 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 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., Reyes and Fetz, 1993; Galán et
al., 2005; Tateno and Robinson, 2007; Phoka et al., 2010) 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; Netoff et al. 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 to 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 ± 3 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 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 towards 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 EPSC 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 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).

Since 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 size 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) normalized to 1 mV. The relationship between EPSP size and membrane potential in the combined data set (Fig. 10D) is significantly less than zero ($p = 0.005$), but the relationship is extremely weak ($r^2 = 0.001$, 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 true of GABAergic IPSPs 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 subthalamic 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 dependence might have introduced complications, appear simply to boost EPSP amplitude within the stimulated ISI and provide a weak exponentially decaying excitation in later ISIs that accounts for most of the stimulus-induced changes in poststimulus ISIs. Although STN cells did deviate from phase model behavior in certain ways, our data suggest that these models are accurate enough to support at least qualitative inferences about the collective behavior of STN neurons.

This is not to say that the iPRC is an adequate description of the neuron for all input conditions. It does not predict the responses of STN neurons to prolonged inhibition that draws the cell out of the voltage range of its autonomous oscillation or the rebound firing that follows such hyperpolarization (e.g. Wilson and Bevan 2011). It also does not predict the responses of neurons to large depolarization that trigger changes in threshold (Farries et al. 2010). Finally, our results do not imply that sequences of synaptic inputs may be treated as the summation of their phase resetting effects even when Equation 1 remains an accurate description of the neuron's response. We show that for most phases, two small synaptic inputs delivered at the same time produce a
phase shift approximately equal to that of a single stimulus with amplitude equal to their sum. But for two stimuli presented at different times in the same interspike interval, the effective phase of presentation of the second is altered by the first. Their effects cannot be treated additively.

If neurons in the external segment of the globus pallidus (GPe) can also be reasonably well represented by phase modes, then this approach might help us understand the dynamics of the reciprocally connected GPe-STN network, whose contribution to basal ganglia function remains unclear. This must await the results of a detailed study of GPe iPRCs, however. In the meantime, we can draw some conclusions about interactions among STN cells. It is not currently clear whether STN cells are synaptically connected with each other—there is some electrophysiological evidence that they are (Shen and Johnson 2006), but the anatomical evidence is equivocal (Kita et al. 1983). If they are interconnected, then their iPRCs can determine whether their interconnections will tend to drive them towards or away from synchronous firing. For example, if a population of cells have PRCs that give only phase advances for excitatory input regardless of input phase (known as "type I" PRCs), then excitatory coupling among these cells often exerts a desynchronizing influence (Ermentrout 1996; Hansel et al. 1995). If, on the other hand, their PRCs predicted phase advances for some input phases and phase delays for others (type II PRCs), then excitatory synaptic coupling could cause activity in this population to synchronize (Hansel et al. 1995). Since STN cells are glutamatergic and all synaptic iPRCs we measured in the STN were resolutely type I, synaptic connections among STN cells could act to desynchronize their activity. In fact, neural activity in the STN in vivo is normally desynchronized (Wichmann et al. 1994), but synchronous oscillations often
appear in Parkinson's disease (Bergman et al. 1994; Levy et al. 2000; Moran et al. 2008; Steigerwald et al. 2008). It is conceivable that a change in iPRC structure contributes to disease-related changes in synchronous activity.

One of our major findings is the large gap between iPRCs estimated from synaptic data and those measured using current pulses. A study of PRCs in pyramidal neurons of cat sensorimotor cortex found no systematic difference between PRCs measured with EPSPs and current pulses when current pulses were adjusted to give the same amount of depolarization as the EPSP (Reyes and Fetz 1993b). It is difficult to guess which findings should be considered more typical, since this kind of quantitative comparison is rare. The difference between synaptic and current pulse iPRCs might be explained by a slow component of the EPSP whose contribution is not accounted for by our measurements of EPSP amplitude, and NMDARs seem a likely source for such a component. However, the NMDAR antagonist APV did not have a significant effect on the gap between synaptic and current pulse iPRCs; the effect of APV on first-order PRCs appears to be mainly limited to a reduction in EPSP size. This result seems to contradict our observations on higher-order iPRCs, where there is clear evidence for slowly decaying excitation that is sensitive to APV. If there is an APV-sensitive slow EPSP, why is its effect not manifest in the first-order iPRC? Here, the voltage-dependence of NMDARs may be playing a role: it is possible that most of the current flows in through NMDA receptors during the fast component of the EPSP, when AMPA-type receptors (AMPARs) provide strong local depolarization at the site of the synapse, or during the action potential when the dendrites may again be strongly depolarized. 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 could 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 basic set of conductances and fall within a single cell type (Bevan and Wilson 1999; Nakanishi et
Clearly, there is some quantitative variability in the expression of those 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.

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Figure 1. Measuring the synaptic iPRC in an example cell. **A,** Example trace showing the regular autonomous activity of this cell. This cell had an autonomous firing rate of 6.6 Hz and ISI CV of 0.06. **B,** Example trace showing an ISI containing an evoked EPSP (black) plotted with example spontaneous ISIs (gray) aligned to the time of the first spike in each ISI. **C,** Total phase shift induced by EPSPs plotted as a function of the phase at which the EPSP arrived. *Black circles* indicate trials in which the entire EPSP fell within the stimulated ISI. *Gray circles* show trials in which a spike was fired during the EPSP; in these cases the stimulated ISI contained only a fraction of the total EPSP. The *solid blue line* marks the maximum possible phase shift given the input phase. **D,** Measuring EPSP amplitude. *Black* trace of the main panel shows average EPSP evoked at input phases of 0.2 - 0.5. The *red* trace plots the average of spontaneous ISIs for each trial, where the average spontaneous ISI for a given trial has been aligned to the first spike of the stimulated ISI for that trial. *Inset,* time derivative of the average EPSP, used to select the time at which the EPSP is judged to be complete. That time is the point at which the rate of rise decayed back to the prestimulus rate of rise (marked here with a horizontal line); the *red circle* marks our judgment of when the EPSP is over in both the inset and main panel. **E,** iPRC for this cell, where total phase shift has been normalized by the average EPSP amplitude to give the phase shift per mV of stimulus-induced potential change. The *gray circles,* denoting trials in which the stimulated ISI contained only part of the EPSP, are normalized only by the fraction of the EPSP amplitude that fell within that ISI. The *red* trace is the result of smoothing these points with a Gaussian filter. **F,**
Threshold of the first poststimulus spike relative to the spontaneous spike threshold, plotted as a function of input phase. Stimuli delivered at late input phases tend to trigger spikes at a lower threshold, and most trials exhibiting lower thresholds are those in which the spike was fired during the EPSP (gray circles).

Figure 2. Overview of synaptic iPRCs recorded in subthalamic cells. A, iPRCs estimated from the response to EPSPs in 89 cells (thin gray traces) plotted with the median synaptic iPRC (thick black trace) and the 25th and 75th percentiles of the iPRC range (thin black traces). B, Individual iPRCs and iPRC quartiles, as in A, including only trials containing the entire EPSP.

Figure 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 EPSPs sizes included in the 4 groups we divide our data into 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 one-way analysis of variance. 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 one cell; the ordinate is the normalized phase shift of that cell's iPRC averaged
over input phases <0.6, while 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 (\(\varphi > 0.85\)).

**Figure 4.** Effect of intrinsic firing rate on synaptic iPRCs. **A**, Histogram of intrinsic
firing rates in our data set. The colored bars shown below the histogram mark the range
of firing rates included in the 4 groups we divide our data into to investigate the effect of
this factor. **B**, Average iPRCs estimated from firing rates of 4 different ranges. **C**,  
Probability of type I error when testing the null hypothesis that the means in these 4
groups are all equal by one-way analysis of variance, like the analysis performed for
EPSP amplitude in Figure 3C. 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 firing rate is
equal to zero; the red line again shows the p = 0.05 criterion. **E**, Relationship between
normalized phase shift and firing rate at relatively early phases. Each point represents
data from one cell; the ordinate is the normalized phase shift of that cell's iPRC averaged
over input phases <0.5, while the abscissa is the intrinsic firing rate of that cell. 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 (\(\varphi > 0.65\)).
Figure 5. Higher-order iPRCs estimated using EPSPs. A, Median second-order synaptic iPRC (thick black trace) plotted with the 25th and 75th percentiles of the second-order iPRC range (thin black traces). B, Effect of EPSP amplitude on second-order iPRCs. Thick traces show the average second-order iPRCs estimated from EPSPs of 4 different amplitude ranges. The EPSP amplitude groups are the same as in Fig. 3A, B: <1.5 mV (black), 1.5 - 2.5 mV (blue), 2.5 - 3.5 mV (green), and >3.5 mV (red). The thin black traces show the mean iPRC for <1.5 mV EPSP ± standard error of the mean, illustrating that is it is not necessarily different from zero (in fact, it is not; see text). C, Effect of firing rate on second-order iPRCs. The average second-order iPRCs for 4 different ranges of firing rate are shown. The firing rate groups are the same as in Fig. 4A, B: <6 Hz (black), 6 - 9 Hz (blue), 9 - 14 Hz (green), and >14 Hz (red). D, Average normalized phase shift as a function of time since the EPSP. Each point represents the average normalized phase shift of all ISIs (taken from all cells combined) that began within a narrow (1 ms wide) range of times since the EPSPs. ISIs that might have contained some of the EPSP (i.e., because the spike defining the start of the ISI was fired before the EPSP was over) are excluded, just as they are excluded from the second-order iPRCs shown in A - C. The red line shows an exponential fit to these data, limited to ISIs initiated >30 ms after the EPSP (this avoids what could be construed as a brief rising phase of this response).

Figure 6. Measuring the current pulse iPRC in an example cell. This is the same cell whose synaptic data are shown in Figure 1. A, Total phase shift induced by current pulses
(each 2 ms in duration) plotted as a function of the phase at which the pulse was
delivered. Red circles show the response to +75 pA pulses, blue circles show the
response to -75 pA pulses. Gray circles show trials in which a spike was fired during the
pulse; in these cases the stimulated ISI contained only a fraction of the total pulse. The
diagonal black line marks the maximum possible phase shift given the input phase. B,
Measuring membrane potential changes caused by current pulses. Black traces show
average membrane potential trajectories following +75 pA and -75 pA pulses delivered at
input phases of 0.2 - 0.5. The red trace plots the average of spontaneous ISIs for each
trial, where the average spontaneous ISI for a given trial has been aligned to the first
spike of the stimulated ISI for that trial. The red and blue circles mark the points at
which the membrane potential changes are measured. The square pulse waveforms
plotted below show the timing and duration of the current pulses that caused the voltage
deflections shown above. C, iPRC for this cell derived from both positive (red) and
negative (blue) current pulses, where total phase shift has been normalized by the pulse-
induced membrane potential change. The gray circles, denoting trials in which the
stimulated ISI contained only part of the current pulse, are normalized only by the
fraction of the pulse that fell within that ISI. The red and blue traces show the smoothed
iPRCs derived from +75 pA and -75 pA current pulses, respectively. The black trace
shows the smoothed iPRC derived from all pulses combined. D, Synaptic (green trace)
and current pulse (black trace) iPRCs measured in this cell, plotted together. The thin
blue trace is the synaptic iPRC obtained by deconvolution of the synaptic current
waveform from the raw synaptic PRC, rather than simply dividing the raw phase shift by
EPSP amplitude (green trace). The thin red trace is the current pulse iPRC convolved
with the normalized synaptic current, illustrating the negligible effect the synaptic current waveform has on the measured iPRC.

Figure 7. Properties of current pulse iPRCs. A, iPRCs estimated from the response to current pulses in 66 cells (thin gray traces) plotted with the median current pulse iPRC (thick black trace) and the 25th and 75th percentiles of the iPRC range (thin black traces). B, Comparison of first-order iPRCs estimated using depolarizing (red) and hyperpolarizing (blue) current pulses in cells where both pulse polarities were used (63 cells). Thick traces show the mean, while the thin traces show the mean ± SEM. C, Comparison of second-order iPRCs estimated using depolarizing (red) and hyperpolarizing (blue) current pulses in cells where both pulse polarities were used, and limited to a subset of data that allowed us to see average second-order iPRCs at relatively late input phases (51 cells). Thick traces show the mean, thin traces show the mean ± SEM. D, Threshold of the first poststimulus spike relative to the spontaneous spike threshold plotted as a function of input phase, with data from positive (red) and negative (blue) current pulses. Thick traces show the mean, thin traces show the mean ± SEM. E, Relationship between normalized phase shift and the relative spike threshold of the first poststimulus spike following positive current pulses, averaged over late input phases ($\phi > 0.8$). Each point represents data from one cell. The regression line for this relationship is shown in red. F, Comparison of iPRCs estimated from EPSPs (green) and current pulses (black). Thick traces show the mean, thin traces show the mean ± SEM.
Figure 8. Effect of APV on iPRCs. A, Synaptic iPRCs measured before (blue) and during (red) bath application of 50 µM APV, n = 14. In panels A - E, thick traces show the mean, thin traces show the mean ± SEM. B, Raw synaptic iPRCs measured before (blue) and during (red) application of APV. These are averages of PRCs that have not been normalized by EPSP size. C, Current pulse iPRCs measured before (blue) and during (red) application of APV, n = 9. D, Pairwise difference between synaptic iPRCs measured in control (blue) or APV (red) and current pulses measured in control conditions, n = 14. Control current pulse iPRCs were subtracted from both control and APV synaptic iPRCs to maximize the sample size (current pulse iPRCs were collected in APV for only 9 cells) and because the purpose was to determine whether NMDAR blockade made synaptic iPRCs more like control current pulse iPRCs. E, Second-order synaptic iPRCs measured before (blue) and during (red) application of APV. F, Average normalized phase shift as a function of time since the EPSP, in control (blue) and APV (red). Each point represents the average normalized phase shift of ISIs starting within a 1 ms time bin. The solid lines show exponential fits.

Figure 9. Examples of individual iPRCs derived from synaptic (thick green trace) and current pulse (thin black trace) data.

Figure 10. Synaptic currents evoked by stimulation in the internal capsule. A, Example of the time derivative of an averaged EPSP (black), proportional to the perisomatic current generated by the synaptic input, plotted with the EPSC recorded in the same cell in voltage clamp (red). The EPSP derivative is plotted on an inverted scale to facilitate
comparison; this EPSP was 3.1 mV. The EPSC is an average of 10 traces, recorded at a
holding potential of -64 mV. B, Histograms of decay time constants for EPSP derivatives
(black, n = 89 cells, bin size 0.2 ms) and EPSCs (red, n = 47 cells, bin size 0.5 ms). One
EPSC measurement (τ = 1.1 ms) is concealed behind the histogram of EPSP derivative
time constants, while another falls outside the range of the plot (τ = 30.8 ms); all the
others (n = 45) are visible. C, Relationship between the size EPSP recorded in current
clamp and integral of the EPSC recorded in voltage clamp. Black circles are from
recordings with low series resistance (<30 MΩ), gray circles are from recordings with
high series resistance (≥30 MΩ), and the red line is the linear fit to all the data. D,
Normalized EPSP amplitude as a function of membrane potential, including only cells
with measurable EPSPs that covered a voltage range that included the reference potential
of -57 mV (n = 66 cells). EPSP amplitudes were normalized to give an average
amplitude of 1 mV at -57 mV (see Methods). Gray circles are individual EPSPs, red line
is the linear fit.
**A**

EPSP Size (mV)

**B**

Input phase

**C**

ANOVA p-value

**D**

Regression p-value

**E**

Phase shift per mV

**F**

Phase shift per mV
AB

decay time constant: 72 ms

0.4 - 1.5 mV
1.5 - 2.5 mV
2.5 - 3.5 mV
3.5 - 5.1 mV
input phase

phase shift per mV

+75 pA
-75 pA

0.2 0.4 0.6 0.8 1

mean ∆V: ±1.41 mV

+1.43 mV
-1.39 mV

2 ms

0.02 0.04 0.06

mean ∆V: ±1.41 mV

+1.43 mV
-1.39 mV

2 ms

0.02 0.04 0.06

synaptic
decomposed synaptic
current pulse
convolved current pulse
input phase | relative spike threshold (mV) | phase shift per mV
--- | --- | ---
0.2 | 0.0 | 0.02
0.4 | 0.2 | 0.04
0.6 | 0.4 | 0.06
0.8 | 0.6 | 0.08
1.0 | 1.0 | 1.0

p = 0.00001, r² = 0.27

input phase > 0.8

A.  
B.  
C.  
D.  
E.  
F.  

positive pulse
negative pulse

synaptic

current pulse
EPSC integral (fC)
normalized EPSP (mV)
decay time constant (ms)
EPSP amplitude (mV)
membrane potential (mV)

2 ms
0.3 V/s (inverted) 6.6 pA
200 400 600 800
-70 -60 -50 -40 2 4 6

< 30 MΩ series R
≥ 30 MΩ series R
p << 0.000001
r² = 0.64

r² = 0.64

normalized EPSP (mV)
membrane potential (mV)