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Robustness, variability, phase dependence, and longevity of individual synaptic input effects on spike timing during fluctuating synaptic backgrounds: A modeling study of globus pallidus neuron phase response properties

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Abstract

A neuron's phase response curve (PRC) shows how inputs arriving at different times during the spike cycle differentially affect the timing of subsequent spikes. Using a full morphological model of a globus pallidus (GP) neuron, we previously demonstrated that dendritic conductances shape the PRC in a spike frequency dependent manner, suggesting different functional roles of perisomatic and distal dendritic synapses in the control of patterned network activity. In the present study we extend this analysis to examine the impact of physiologically realistic high conductance states on somatic and dendritic PRCs and the time course of spike train perturbations. First, we found that average somatic and dendritic PRCs preserved their shapes and spike frequency dependence when the model was driven by spatially-distributed, stochastic conductance inputs rather than tonic somatic current. However, responses to inputs during specific synaptic backgrounds often deviated substantially from the average PRC. Therefore, we analyzed the interactions of PRC stimuli with transient fluctuations in the synaptic background on a trial-by-trial basis. We found that the variability in responses to PRC stimuli and the incidence of stimulus-evoked added or skipped spikes were stimulus-phase-dependent and reflected the profile of the average PRC, suggesting commonality in the underlying mechanisms. Clear differences in the relation between the phase of input and variability of spike response between dendritic and somatic inputs indicate that these regions generally represent distinct dynamical subsystems of synaptic integration with respect to influencing the stability of spike time attractors generated by the overall synaptic conductance.

Keywords

phase response curve (PRC); high conductance state; stochastic synaptic background; spike time attractor; dendrite; SK current; synchronization; oscillation

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Introduction

Neurons *in vivo* are subject to a constant barrage of excitatory and inhibitory synaptic inputs that are distributed throughout the neuronal morphology, thus putting the neuron in a state of high membrane conductance. Such high conductance states (Destexhe and Pare, 1999, Rudolph and Destexhe, 2001, Destexhe et al., 2003, Rudolph and Destexhe, 2003a, b) are capable of switching the class of excitability by which a neuron initiates action potentials (Hodgkin, 1948, Prescott et al., 2008), the resonant properties of a neuron (Fernandez and White, 2008), and can affect conductance-based mechanisms of synaptic integration (Rudolph and Destexhe, 2001).

In neuronal systems the phase response curve (PRC) describes how inputs to a neuron at different times during the spike cycle affect the timing of subsequent spikes (Winfree, 2001, Smeal et al., 2010, Schultheiss, 2012b). Type I PRCs are composed predominantly of positive values indicating that depolarizing inputs nearly always advance the timing of the next spike, whereas type II PRCs contain a significant negative lobe indicating, paradoxically, that *excitatory* inputs can *delay* spiking when delivered at some phases (typically early phases) of the spike cycle. PRC shape is dependent on the species and spatial distribution of membrane currents that a neuron possesses (Gutkin et al., 2005, Goldberg et al., 2007, Stiefel et al., 2008, Stiefel et al., 2009, Schultheiss et al., 2010, Ermentrout et al., 2012, Schultheiss, 2012a) as well as on the neuron's spike frequency (Gutkin et al., 2005, Schultheiss et al., 2010, Schultheiss, 2012a), and PRC shapes reflect neuronal excitability dynamics (Hodgkin, 1948, Rinzel and Ermentrout, 1998, Brown et al., 2004) and modulation state (Stiefel et al., 2008, Stiefel et al., 2009, Stiefel and Gutkin, 2012). PRCs are also powerful and efficient predictors of patterned network behavior, and type II PRCs are typically well suited to support synchronization phenomena in coupled networks (Hansel et al., 1995, Ermentrout, 1996, Acker et al., 2003) and entrainment of spiking to fluctuating or oscillatory input patterns (Rinzel and Ermentrout, 1998, Galan et al., 2006, 2007a, b, Marella and Ermentrout, 2008, Abouzeid and Ermentrout, 2009). Weak-coupling within a neural network is only sufficient to achieve synchronization if the individual oscillators have similar frequencies (Ermentrout and Kopell, 1991, Dodla and Wilson, 2009), but strong-coupling can result in destabilization of phase locked states under some conditions (Ermentrout and Kopell, 1991, Oh and Matveev, 2009). Furthermore, phase dependent variance in the PRC can affect the degree and stability of network synchrony (Ly and Ermentrout, 2010, Ermentrout et al., 2011). However, PRC analysis can be used to predict near synchronous states under strong-coupling conditions (Maran and Canavier, 2008), and synchronization resulting from type II phase response dynamics can generalize to the strong-coupling case (Bogaard et al., 2009). To date, the effects on PRCs of high conductance states and high spike time variability due to considerable synaptic input fluctuations characteristic of *in vivo* conditions have not been addressed.

In the present study we evaluated how high conductance states composed of fluctuating synaptic background activity influence the effects of additional single synaptic inputs on spike timing using measures of spike train perturbations, including PRCs, from simulations with different background characteristics and input locations. First, we extended our previous analysis of a morphologically reconstructed globus pallidus (GP) neuron model (Schultheiss et al., 2010) to address the consequences of high conductance states for neuronal PRCs. Having previously demonstrated that dendritic SK current can cause type II PRCs for dendritic inputs even when the somatic PRC is type I (Schultheiss et al., 2010, Schultheiss, 2012a), we specifically investigated whether this mechanism can persist in the high-conductance state. Using average PRCs derived across many trials with each of several synaptic backgrounds spanning the physiological range, we tested how driving the model with stochastic spatially-distributed conductance backgrounds, rather than with tonic

somatic current, affects the shape and spike frequency dependence of somatic and dendritic PRCs.

In the second part of this study, we analyzed on a trial-by-trial basis how phasic synaptic input can perturb spike timing away from attractors generated by the stochastic background input patterns. To accomplish this we used several measures of spike timing perturbations including the incidence and phase dependence of added or skipped spikes elicited by PRC stimuli, the extent to which average PRCs were able to predict input effects in individual trials, i.e. PRC variance, and the longevity across spike cycles of perturbations of spike timing. Finally, we developed a new type of PRC plot, the cumulative PRC, for describing the phase response properties of neurons during high conductance states. The cumulative PRC captures how perturbations of spike timing evolve across subsequent spike cycles and distinguishes intrinsic effects from the effects of synaptic background activity.

Experimental Procedures

All simulations were run using the GENESIS software platform (www.genesis-sim.org/GENESIS) on Emory University High Performance Compute Clusters (Sun Microsystems). A fixed time step of 20 μ s was used for all simulations using the implicit Crank-Nicholson integration method. Custom Matlab (The MathWorks, Natick, MA) routines were used for analysis of voltage, current, conductance, and spike time data.

In order to address theoretical propositions from the PRC literature based largely on analyses of reduced models or experimental preparations under highly idealized circumstances, we incorporated considerable physiological realism both in the GP neuron model itself (Hanson et al., 2004, Gunay et al., 2008, Edgerton et al., 2010, Schultheiss et al., 2010, Edgerton and Jaeger, 2011) and in the synaptic backgrounds applied to it, as described in the following sections.

GP Neuron Model

Morphology and Passive Electrical Properties—The morphology and passive electrical properties of the baseline model (GP_{base}) have been previously described in detail (Hanson et al., 2004, Gunay et al., 2008). In brief, using NeuroLucida (MicroBrightField, Inc.) we reconstructed the morphology of a GP neuron for which a battery of electrophysiological recordings had been made and created a GENESIS morphology file using CVAPP software (www.compneuro.org). Passive biophysical parameters of the model were set so that voltage responses to current injection stimuli reproduced those measured in the recorded neuron (Hanson et al., 2004). To allow axonal spike initiation and realistic axonal current sources and sinks, a standard axon consisting of a highly excitable axon initial segment and nodes of Ranvier separated by myelinated inter-node segments, was adapted from Shen et al. (1999) and attached to the soma (Gunay et al., 2008).

Active Conductances and Model Tuning—One calcium-activated conductance, the small-conductance calcium activated potassium current (SK), modeled using the Hill equation, and eight voltage-gated membrane conductances, modeled using the Hodgkin-Huxley formalism, were implemented in the model based on experimental evidence of their presence in GP neurons. The voltage-gated conductances include: fast-transient and persistent sodium currents, NaF & NaP; fast and slow delayed-rectifier (K_{dr}) potassium currents, K_{v3} & K_{v2}; an A-type potassium current, K_{v4}; an M-type potassium current, KCNQ; the hyperpolarization-activated mixed cation current, or h-current; and a high-voltage-activated calcium current (Ca_{HVA}) representing a mixture of L-, N-, and P/Q-type currents and which acts as the calcium source for SK activation.

Each conductance was distributed uniformly throughout the dendrite with the exception of Ca_{HVA} , which had a greater density in thinner, distal dendritic compartments (Hanson and Smith, 2002). During the tuning process, conductance densities were determined using a semiautomated process comparing model behaviors with physiological recordings, and a thorough exploration of parameter space was performed (Gunay et al., 2008, Schultheiss et al., 2010). As described in our previous studies, GP_{base} sits within a wide parameter basin across which considerable parameter variations result in smoothly varying electrophysiological features and falls well within the physiological variability for the following electrophysiological measures: spontaneous spike waveform, spontaneous spike frequency, somatic FI curve, spike frequency adaptation, spike height attenuation during positive current steps, voltage ‘sag’ during negative current steps, and latency to the first spike following the offset of negative current steps. The full GENESIS model is available from ModelDB (<http://senselab.med.yale.edu/ModelDB/>). In order to specifically test the role of dendritic SK in shaping responses to phasic synaptic stimuli, we also generated a version of the model lacking dendritic SK conductance (GP_{NDSK}).

PRC Analysis During High Conductance States

Synaptic backgrounds—In order to generate spiking in the model that reproduced many of the essential features of GP neuronal activity *in vivo*, it was necessary to determine how synaptic background parameters influenced the frequency (Fig. 1) and regularity of spiking (Fig. 2). The synaptic backgrounds were composed of individual dual exponential conductance injections representing excitatory AMPAergic synaptic inputs (1 ms rise time, 3 ms decay time, $E_{\text{AMPA}} = 0$ mV) or inhibitory GABA_A synaptic inputs (0.8 ms rise time, 5 ms decay time, $E_{\text{GABA}} = -80$ mV) at 100 AMPA synapses and 1022 GABA synapses distributed randomly throughout the dendrite. These values reflect the approximate proportion and kinetics of synaptic inputs to GP from the subthalamic nucleus (STN) (Robledo and Feger, 1990, Hamada and DeLong, 1992, Shink and Smith, 1995, Nambu et al., 2000, Kita et al., 2005) and striatum (Tremblay and Fillion, 1989, Shink and Smith, 1995, Sims et al., 2008).

The base GP model, which spikes spontaneously with a 7.9 Hz oscillation (Schultheiss et al., 2010), was driven to spike at *in vivo* frequencies with a variety of synaptic background parameter sets determined by exploration of the parameter space for maximal unitary conductance (gain) and input frequencies of synaptic inputs. Figure 1A shows for GP_{base} , the set of iso-frequency lines describing the balances between excitatory and inhibitory input frequencies that yielded target output spike frequencies of 15 Hz, 30 Hz, and 45 Hz for each synaptic gain level. Excitatory input frequencies necessary to drive output spiking to the target frequencies were interpolated for all combinations of 3 synaptic gain levels (0.5 nS, 1 nS, and 2 nS peak unitary conductance) and inhibitory input frequencies of 0.5 Hz and 5 Hz. (We refer to the synaptic backgrounds with 5 Hz inhibition as ‘high input frequency’ backgrounds.) The resultant excitatory input frequencies were realistic for STN neurons *in vivo*, and the inhibitory input frequencies chosen sample broadly from the range expected for striatal neurons during up states and down states supported by periods of sustained or diminished cortical and thalamic excitation (Wilson and Groves, 1981, Onn et al., 1994, Wilson and Kawaguchi, 1996, Chen et al., 2001). Note that although individual striatal synapses have relatively low activation rates, there is considerable convergence of inputs from many different striatal neurons onto individual GP neurons, as is captured by the high proportion of inhibitory synapses participating in the synaptic backgrounds which we applied to the GP model. The process of interpolating excitatory input frequencies to achieve the target output spike frequencies with 0.5 Hz inhibitory input was repeated for the GP_{NDSK} model (Fig. 1B&D). In the absence of the strong outward dendritic SK current,

lower excitatory input frequencies were able to drive the GP_{NDSK} model to the target spike frequencies than were required for the GP_{base} model.

The synaptic background parameter sets sample thoroughly from a wide parameter space of different input conditions, thus providing a diversity of biologically plausible, high conductance states that represent a first approximation of the *in vivo* inputs to GP neurons appropriate for our analyses. The notable exception to the *in vivo* realism of the synaptic backgrounds generated in this manner is that the timing of synaptic inputs is purely stochastic, rather than possessing correlated patterning. The necessary experimental data to describe correlations among neurons within each structure projecting to GP and between those structures (striatum, STN, and intrinsic projections within GP) is not known in sufficient detail to fully recreate this level of structure in our simulations. Thus, our synaptic backgrounds are approximated embodiments of the *in vivo* 'state' of the neuron, and provide an essential foundation for further investigations of structured synaptic activity patterns and *in vivo* control of spike timing. For each synaptic background parameter set we then generated 100 control spike trains differing only in the random timing of the individual synaptic inputs. These control spike trains were essentially spike time attractors determining the timing of spike events triggered by the fluctuations in synaptic background activity. Using these same sets of attractors in repeated simulations (similar to frozen noise), we then assessed the effect of additional phasic synaptic inputs on the subsequent spiking patterns.

PRC Simulation and Analysis Protocols—To allow the model to adapt to the ongoing synaptic backgrounds before conducting PRC analysis, we simulated a 10 s period for each background without additional PRC stimuli. To avoid repeating these simulations of adaptation time for each PRC simulation, we saved all state variables in a 'snapshot' file after conducting these simulations once, and used them for subsequent PRC simulations. Next, one hundred single-trial PRCs were generated for each synaptic background parameter set by delivering additional excitatory PRC stimuli at different phases of the first inter-spike interval (ISI) of each of the 100 control spike trains. For each trial, in 72 separate simulations, a 2.5 nS AMPA input representing activation of one or a few excitatory synapses (Hernandez et al., 2006) was delivered at one of 72 evenly-distributed time points. The onset of the earliest input was timed to be coincident with the somatic spike delineating the start of first spike cycle. Inputs were delivered either to the soma or to a distal dendritic region (Fig. 3A) where the 2.5 nS conductance was evenly divided among synapses on 25 contiguous compartments. Spike times were recorded using the GENESIS spikehistory element with a precision of 20 μ s, and input-evoked shifts in spike timing were calculated relative to the spike terminating the first control spike cycle. Primary (first order, or F1) PRCs were plotted as spike advances (in units normalized to the period of the control ISI, i.e. % of phase) as a function of input phase, such that positive values reflect advancements of the spike cycle. Higher-order PRCs (F2, F3, etc.) describing the effects of stimuli on subsequent spike cycles were normalized to the respective spike cycles.

Our first goal in this study was to determine whether PRCs obtained during *in vivo* high conductance states would maintain the prominent features and spike frequency dependence of those derived during pacemaking of the model. For comparison with PRCs during pacemaking at each spike frequency, we averaged single trial PRCs for each synaptic background parameter set and stimulus location (F1 and F2 are illustrated). We further analyzed the effect of spike frequency on PRC shape *within* parameter sets for synaptic backgrounds composed of 0.5 nS unitary inputs, by deriving average PRCs for subsets of trials with the 25 fastest, 25 slowest, and 50 intermediate interspike intervals among the 100 trials for each mean spike frequency (corresponding to the right tail, left tail, and center of each ISI distribution shown in Fig. 2A, dotted lines) (Supp. Fig. 1). In this analysis, the mean synaptic background activity and mean output spike rates were identical between

subsets of trials, and only the random *momentary* input fluctuations and *instantaneous* spike frequencies differed.

Our second goal in this study was to characterize the variability and longevity of perturbations of the spike pattern by phasic PRC stimuli. Here, our approach was to view the control spike patterns driven by ongoing stochastic synaptic activity as spike time attractors, which were temporarily disrupted by a single phasic PRC stimulus in each simulation. By varying the parameters of the synaptic backgrounds we were able to vary the strengths of the spike time attractors, and we then evaluated the spike rate dependence, stimulus phase dependence, and stimulus location dependence of spike timing perturbations in terms of variability across trials and longevity across spike cycles as follows: We first defined 3 types of events that were commonly observed as a consequence of interactions between PRC stimuli and fluctuations in the ongoing synaptic backgrounds: added spikes, skipped spikes, and divergence events. Added spikes were instances where spike trains for simulations containing a PRC stimulus contained a spike that was not present in the control simulation without the PRC stimulus, and skipped spikes were instances where the control spike train contained a spike that was not present in simulations containing a PRC stimulus. The effect on the spike train of adding a PRC stimulus on top of ongoing synaptic backgrounds often lasted for many spike cycles, but eventually most perturbations diminished and the timing of subsequent spiking returned to that of the control spike train. We defined ‘convergence’ as the process by which perturbations diminished across successive spike cycles, and deemed convergence to be complete when the average difference between spike times in the 72 stimulated simulations for each trial was less than 0.5 ms from the control spike times for that trial. In some cases, however, the spiking trajectory in perturbed simulations met our criterion for having converged only to ‘diverge’ again and deviate from the control spike train. We defined such instances as ‘divergence events.’ Each of these types of events was identified using a custom automated algorithm and the phase of stimuli leading to these events as well as the spike cycle in which they occurred were recorded for further analysis.

To analyze the variability across trials of the effect of PRC stimuli on spike timing, we introduce phase response-variance curves (PRVCs), which plot the phase dependence of the variance of spike shifts for each synaptic background across spike cycles. Using direct measurement of spike shifts and PRC variance amidst ongoing synaptic fluctuations like those expected under *in vivo* conditions, this approach differs from previous methods of fitting *in vitro* experimental data during pacemaking (Netoff et al., 2005, Ermentrout et al., 2011). Making use of spatially-distributed, physiological-sized unitary inputs (exceeding weak-coupling limitations) to compose synaptic backgrounds, our approach provides a more realistic estimate of expected variability in responses of GP neurons to excitatory inputs *in vivo*.

We characterized the progression of convergence as a function of synaptic background parameters in order to provide a profile of the temporal windows within which synaptic inputs can influence subsequent spike timing under a variety of high conductance conditions. For each synaptic background parameter set, we plotted the proportion of trials that remained unconverged as a function of the number of spike cycles that had elapsed since stimulus delivery. Trials containing added spikes, skipped spikes, or divergence events, as well as trials in which perturbed simulations did not meet our criterion for convergence within 20 spike cycles following stimulation (unconverged trials) were excluded from the population of trials used for this analysis and also did not contribute to the average PRCs shown later.

To allow intrinsic effects on spike timing evoked by PRC stimuli to be readily distinguishable from the effect of synaptic backgrounds (driving spikes back onto the

control trajectory by the process of convergence) we introduce cumulative PRCs which plot for each spike cycle the cumulative effect of perturbations on spike timing assessed at the time of each successive spike in the spike train. Thus, the C1 PRC is the same as the average F1 PRC, because only one spike cycle is being considered. The C2 PRC is the sum of the average F1 PRC and the average F2 PRC, the C3 PRC is the sum of average F1, F2, and F3 PRCs, and so on. By plotting cumulative effects on spike timing in this way, changes in the shape of cPRCs or vertical translation of cPRCs reflect the contribution of active intrinsic mechanisms to spike timing, whereas attenuation of cPRCs reflects the convergence of perturbed spike trains back to the control spike train across successive spike cycles.

Results

Robustness of somatic and dendritic PRCs to stochastic synaptic background activity

High conductance states generated by ongoing synaptic activity *in vivo* pose at least three major challenges to the pattern of somatic type I and dendritic type II phase response properties we have previously described for model GP neurons (Schultheiss et al., 2010, Schultheiss, 2012a): 1) because the types of neuronal PRCs are related to the bifurcation underlying spike initiation, high conductance states could switch somatic PRCs for the GP model between type I and II by shifting spike threshold and/or activating additional membrane conductances in the near-threshold voltage range (Prescott et al., 2006, 2008); 2) the elevated membrane conductance throughout the neuronal morphology that accompanies a high level of synaptic input reduces the neuron's input resistance and could greatly attenuate the impact of dendritic inputs on spike timing; and 3) the interaction of PRC stimuli with transients in the synaptic background and local active intrinsic properties could yield nonlinear effects on spike timing that are highly variable across trials and potentially overwhelm the prominent features in somatic and dendritic PRCs obtained during spontaneous pacemaking. We first describe the generation of high conductance states in our GP model by application of stochastic synaptic backgrounds, and then we address the challenges posed by the high conductance state to PRC analysis.

Generation of high conductance states with stochastic synaptic backgrounds

—In order to simulate the likely base condition of *in vivo* GP firing behavior, we imposed a background of randomly-timed, spatially-distributed excitatory (AMPA) and inhibitory (GABA) synaptic inputs to the model (see Experimental Procedures for details). We varied the unitary conductance (synaptic gain) and frequency of background synaptic inputs across the likely physiological range to generate output spiking of the model at frequencies matching those observed during *in vivo* recordings (15–45 Hz) (Mallet et al., 2008) and to control the balance between intrinsic and synaptic drives contributing to spike timing. For each stochastic background, transient imbalances between excitation and inhibition caused the sub-threshold voltage to fluctuate, and higher-gain synaptic backgrounds drove larger sub-threshold voltage fluctuations, more threshold crossings, and thus higher output spike frequencies (Fig. 1) as well as more irregular spiking (Fig. 2). Conversely, low- and mid-gain synaptic backgrounds required higher excitatory input frequencies to achieve the target output spike frequencies than did the high-gain backgrounds (Fig. 1A). As we have recently reported, dendritic SK conductance contributes to the intrinsic spiking rhythmicity of the model (Schultheiss et al., 2010, Edgerton and Jaeger, 2011), and removal of dendritic SK (GP_{NDSK}) in these simulations increased inter-spike interval variance (Fig. 2D&E) although to a lesser degree than increasing synaptic background gain. For PRC analysis, we selected 9 synaptic background parameter sets for GP_{base} (representing all combinations of 3 synaptic gain levels and 3 target output spike frequencies) for inhibitory input frequencies of 0.5 Hz and 5 Hz (Fig. 1A; filled circles). We also selected 9 synaptic background parameter sets for the GP_{NDSK} model for the lower level of inhibition (Fig. 1B, filled circles).

Somatic and dendritic PRCs during pacemaking—As we have previously shown during intrinsic pacemaking, the spatial distribution of the SK current and its calcium source in the GP model, the high-voltage activated calcium current (Ca_{HVA}), create a gradient of PRCs ranging from strongly type I at the soma to strongly type II at the distal dendrite (Schultheiss et al., 2010, Schultheiss, 2012a). PRCs derived during oscillatory spiking for inputs to the soma and a representative distal dendritic site (DD in Fig. 3A) are shown in Figure 3B. Furthermore, during faster spiking driven by tonic current injection to the soma, the delaying effect of dendritic SK current impinges more strongly on subsequent spike cycles such that the corresponding negative regions in dendritic PRCs occur in the high-order PRCs (Fig. 3B2.)

Average somatic and dendritic PRCs during high conductance states—During high conductance states, spike timing is influenced by both intrinsic and synaptic currents, and GP neurons *in vivo* are not on a stable oscillatory limit cycle as during intrinsic spiking. The first major goal of this study was to determine whether PRCs derived during high conductance states retain the features of those derived while the neuron oscillates due to intrinsic pacemaking, or if the phase response properties of the model are qualitatively changed by ongoing synaptic background activity, obscuring how the PRCs from isolated neurons should be used to predict synchronized or oscillatory network activity *in vivo*.

For each synaptic background parameter set, 100 control spike trains were generated differing only in the random seeds used to determine the timing of synaptic background input patterns. PRCs were simulated by delivering a single 2.5 nS AMPA-synaptic input to either the soma or the distal dendrite at each of 72 regularly spaced time points (in separate simulations) within the first spike cycle of each of the 100 control spike trains. For each control spike train, the PRC was obtained by measuring the shifts in the post-stimulus spike times (compared to the control spike train) caused by each PRC stimulus. Because the spike pattern during the control simulations was driven by the fluctuating synaptic background that differed for each of the 100 random seeds used, the effects of the additional PRC stimuli were quite variable across seeds resulting in highly variable single trial PRCs. To assess the average effect of phasic PRC inputs on spike timing, we generated average somatic and dendritic PRCs across the 100 seeds. We constructed the average PRC for each simulation condition to evaluate the dependence of PRC shape on stimulus location, spike frequency, dendritic SK conductance, and synaptic background parameters.

Average somatic and dendritic PRCs for GP_{base} during ongoing synaptic background activity (Fig. 3C–F) closely resembled those derived during intrinsic pacemaking (Fig. 3B), particularly when the synaptic backgrounds were relatively weak. This result indicates that the high conductance state did not change the basic spiking mechanism of the model, i.e. the bifurcation leading from quiescence to spiking. For low-gain synaptic backgrounds (Fig. 3C), the average effect across trials of excitatory somatic stimulation was to advance the first spike subsequent to the stimulus (Fig. 3C1; F1). These advances were diminished in the second spike cycle, corresponding to negative values in the second-order PRC (F2). Similar to the oscillating GP_{base} model driven to the same frequencies by applied current (Schultheiss et al., 2010), the somatic PRC showed only slight changes across mean spike frequencies of 15, 30, or 45 Hz driven by different rates of excitatory input (Fig. 3D1). Further analysis of average PRCs derived for subsets of trials with stimulated ISIs of different duration *within* identical synaptic background conditions (described in Experimental Procedures) also showed relatively little effect of the *instantaneous* spike frequency on somatic PRCs, except that the primary (F1) somatic PRCs were somewhat attenuated for stimuli delivered during faster interspike intervals (Supp. Fig. 1A1, B1, & C1).

Average PRCs for stimulation of the dendritic site, however, showed a strong dependence on spike frequency (Fig. 3C2). During 15 Hz spiking, there was a pronounced negative region early in the F1 PRC (green arrowhead) and a late-phase negative peak in the F2 PRC (blue arrowhead). During 30 Hz spiking, the negative regions of the F1 and F2 PRC were somewhat reduced, whereas at 45 Hz the negative peak in the F1 PRC was nearly absent and the negative peak in the F2 PRC occurred earlier in phase. We have previously demonstrated that this pattern of spike-frequency dependence of distal dendritic PRCs is a consequence of the time-course of SK current elicited locally by dendritic PRC stimuli, which at higher spike frequencies impinges less on the stimulated spike cycle and increasingly on the subsequent spike cycle (Schultheiss et al., 2010). For the mean spike frequency of 30 Hz, the shape of dendritic PRCs was also dependent on the *instantaneous* spike frequency (Supp. Fig. 1B2). As described in Experimental Procedures, for this analysis average PRCs were derived for subsets of trials where the stimulated interspike interval was slower than, near, or faster than the mean interval (corresponding to the right tail, center, and left tail of the ISI histograms depicted in Fig. 2). The dependence of dendritic PRC shape on instantaneous spike frequency within the 30 Hz synaptic background condition (Supp. Fig. 1B2) mirrored the spike frequency dependence across synaptic background conditions with different excitatory input rates (Fig. 3C2): The average dendritic PRC for the slowest intervals during 30 Hz mean firing (Supp. Fig. 1B2, blue trace) resembled the average dendritic PRC for the corresponding 15 Hz condition (Fig. 3C2, blue trace) with a negative region early in phase of the F1 PRC and a prominent negative peak at late phases of the F2 PRC. The average dendritic PRC for the fastest intervals during 30 Hz mean firing (Supp. Fig. 1B2, red trace) resembled the average PRC from the 45 Hz condition (Fig. 3C2, red trace), exhibiting a reduced range of phases in the stimulated spike cycle where inputs resulted in phase delays and a left-shifted negative peak in the F2 PRC. However, within the 30 Hz synaptic background condition, the average dendritic PRC for faster intervals exhibited a larger positive peak (Supp. Fig. 1B2), whereas the positive peak in the F1 dendritic PRC was reduced at higher mean spike frequencies driven with greater excitatory input (Fig. 3C2). No clear dependence of dendritic PRC shape on instantaneous spike frequency was observed among subsets of trials where the mean spike frequency was 15 Hz or 45 Hz (Supp. Fig. 1A2&C2), suggesting that the distribution of ISIs surrounding the mean spike frequency observed *in vivo* (30 Hz) is centered on the range where the relatively fixed time-course of dendritic SK can *differentially* affect the timing of the next few spikes following dendritic input. Thus, the shape of dendritic PRCs is selectively sensitive to instantaneous spike frequency during spiking at the mean *in vivo* frequency, because the time-course of dendritic SK activation is of a similar interval length as the spiking interval.

PRCs for ‘higher’ conductance states—The defining features of average PRCs for somatic and distal dendritic stimulation were also robust for the ‘high input frequency’ synaptic backgrounds (Fig. 3D; green and blue arrowheads) and for the mid-gain synaptic backgrounds (Fig. 3E; green and blue arrowheads). With these higher conductance states, however, the membrane voltage fluctuations during the control trials were accentuated, thus leading to greater variability in the effects of PRC stimuli on spike timing and greater standard error surrounding the average PRCs. For the synaptic backgrounds with the highest gain or with both high gain and high input frequency (not shown), average PRCs for distal dendritic inputs were significantly attenuated by the elevated conductance of the dendrite, and the peaks of average PRCs were not as well defined against the fluctuations caused by greater variability across trials. Thus, strengthening synaptic background activity lessened the relative contribution of intrinsic mechanisms to the control of spike timing. This reduction in the amplitude of average PRCs was likely due to the lowered input resistance of the model in the high-conductance state which led to smaller EPSPs resulting from our PRC stimuli and greater shunting of stimulus currents. It is important to note, however, that only

a single additional synaptic input (at each time-point in the stimulated spike cycle) was used as the PRC stimulus, and that stronger synchronous inputs at many synapses are likely to occur under some *in vivo* conditions, including for example, β -frequency rhythmic network activity in Parkinsonian basal ganglia nuclei.

Dendritic SK conductance shapes somatic and dendritic PRCs—The characteristic type II PRCs for dendritic inputs to the GP model were found to be a consequence of dendritic SK conductance which creates a net current sink in the dendrite when activated by excitatory input (Schultheiss et al., 2010). To clarify the role that SK conductance plays in shaping the responses of GP neurons to dendritic input during high conductance states, we examined the shape of average dendritic PRCs for the GP_{NDSK} model. At 30 Hz the removal of SK from the dendrite accentuated advancements of the first spike cycle by somatic stimuli and yielded a greater positive peak in the F1 somatic PRC (Fig. 3F1). The early-phase negative peak in the corresponding F2 PRC was accentuated in tandem with the positive peak in the F1 PRC, reflecting that the larger advancements of the first spike cycle elicited by PRC stimuli were greatly offset by larger delays in the second spike cycle. As we have previously demonstrated for the model during intrinsic pacemaking, this effect is a consequence of *somatic* SK which was left intact in the GP_{NDSK} model (Schultheiss et al., 2010).

Stimulation of the distal dendrite of the GP_{NDSK} model yielded average PRCs that resembled somatic average PRCs (Fig. 3F2). The negative regions occurring early in the F1 PRC and late in the F2 PRC for the GP_{base} model were abolished by the removal of dendritic SK conductance (Fig. 3F2; red arrowhead). The distal dendritic PRC for GP_{NDSK} exhibited a larger positive peak in the F1 PRC and a negative peak in the F2 PRC at a corresponding phase, again indicating that advancements elicited in the stimulated spike cycle were offset by delays in the subsequent spike cycle.

Taken together, average PRCs derived by the application of single additional synaptic inputs amidst ongoing synaptic background activity illustrate the dependence of PRC shape on: 1) the site of stimulation within the neuronal morphology, such that somatic PRCs were type I whereas distal dendritic PRCs were type II, 2) the spike frequency, such that at higher spike frequencies the delaying effect of SK conductance impinged predominantly on the second spike cycle subsequent to stimulation, and 3) the presence of SK conductance in the dendrite. Thus, the effects of individual synaptic inputs on spike timing which we have previously described (Schultheiss et al., 2010, Schultheiss, 2012a) are not limited to the intrinsic oscillatory state but are also likely to shape the responses of these neurons *in vivo*.

Spike train perturbations by phasic synaptic input and interactions with ongoing synaptic background activity

The second major goal of this study was to characterize the effects of individual synaptic inputs arriving at different phases of the spike cycle on the subsequent spike pattern when, as it is *in vivo*, that pattern is itself driven by an ongoing barrage of thousands of other synaptic inputs. Our approach builds on the seminal work of Mainen & Sejnowski (1995) wherein repeated applications of the same ('frozen') fluctuating input to a neuron resulted in markedly similar patterns of output (Mainen and Sejnowski, 1995). Such a fluctuating input pattern, consisting of filtered current noise in the case of Mainen & Sejnowski (1995), approximates the activation of a large number of excitatory and inhibitory synapses, and can be viewed as a spike time attractor which causes the neuron to spike reliably during different stimulus repetitions at times when sufficiently depolarizing fluctuations occur. In our simulations, each control spike pattern can be understood as such a spike time attractor whereby spike events were triggered when the temporal convergence of synaptic

background inputs resulted in sufficiently depolarizing membrane potential fluctuations. Therefore, the addition of individual synaptic inputs to the synaptic background activity can be viewed as perturbations of the spiking pattern away from the control attractor. To directly assess the importance of input phase on subsequent spiking under these conditions, we delivered synaptic perturbations (PRC stimuli) at different phases of the first spike cycle in each control spike train, i.e. on top of frozen patterns of stochastic synaptic inputs for each trial; and to determine how characteristics of the spike time attractor itself influence the perturbations of spiking by phasic synaptic inputs, we used synaptic backgrounds with different input gain and frequency parameters to drive control spiking at different frequencies as described in the methods. As follows, we first analyzed individual cases of spike pattern perturbations which resulted in either smooth return of spiking across spike cycles back to the attractor or highly nonlinear interactions between PRC stimuli and synaptic backgrounds including added or skipped spikes. We then analyzed the variability in spike time shifts evoked by PRC stimuli by generating phase response variance curves for each synaptic background condition. Lastly, we analyzed how the parameters of synaptic backgrounds determined the return of spiking to the attractor pattern, i.e. the decay of synaptic perturbations during each synaptic background condition.

Examples of control (attractor) spike trains perturbed by additional PRC stimuli

—Although average PRCs derived during high conductance states reproduced the dynamics of GP neuron models during intrinsic spiking and are likely to characterize the average behavior of populations of GP neurons, there was considerable variability across trials with each synaptic background random seed. To characterize on a trial-by-trial basis the ways in which phasic synaptic inputs used for PRC analysis interacted with transients in the fluctuating synaptic backgrounds, we compared spike trains perturbed with PRC stimuli to their respective control spike trains and assessed the fate of the perturbation over successive spike cycles.

Figure 4A–D illustrates how PRC stimuli perturbed the spike pattern away from sample control voltage traces (thick black lines) for low-gain (A&B) and high-gain (C&D) synaptic backgrounds. In the low-gain example, somatic stimuli at all phases of the first spike cycle advanced the subsequent spike (Fig. 4A). These advancements of the spike train diminished over the next several spike cycles (corresponding to negative values in the higher order PRCs) until each of the perturbed spike trains had converged back to the control spiking pattern driven by the synaptic background for this trial. Stimuli delivered to the distal dendrite either advanced or delayed the next spike depending on the phase of the stimulus (Fig. 4B), and these shifts away from the control spike train also generally diminished over successive spike cycles. Thus, for fixed patterns of synaptic background inputs (similar to frozen noise), the perturbing effects of additional phasic synaptic inputs were transient, and after some number of spike cycles (which varied widely from trial to trial) the stochastic background regained dominance in determining the timing of subsequent spikes and the remainder of the control spike train was restored. When delivered against higher-gain synaptic backgrounds, somatic or distal dendritic stimuli typically perturbed the voltage trajectory of the model from the control spike trains for fewer spike cycles (Fig. 4C&D) than when the synaptic background was weaker (Fig. 4A&B). Thus, synaptic background strength was a major determinant of the longevity of perturbations initiated by PRC stimuli delivered against ongoing stochastic synaptic activity.

Perturbed spike trains exhibit added spikes, skipped spikes, and divergence events

—The examples shown in Figure 4A–D illustrate relatively smooth convergence of the perturbed voltage trajectories back to the control spike train across successive spike cycles. In many trials, however, the convergence was not smooth and the single-trial PRC had little resemblance to the average PRC. Among these were trials in which PRC stimuli

led to either a gained or a lost spike relative to the control spike train. Examples of such ‘added’ or ‘skipped’ spikes are shown in Figure 4E&F, respectively. As illustrated, it was common for added and skipped spikes to be the result of a complex sequence of events initiated by the PRC stimulus, rather than being triggered directly by the stimulus itself. Figure 4G shows a case where an extra spike was added several spike cycles after stimulation and after the perturbed spike trains had seemed to converge back to the control trajectory (first dashed box), and a subsequent control spike was skipped 6 spike cycles later (second dashed box). This example highlights how slight differences in spike timing can cause the model to respond very differently to transient components of the fluctuating synaptic background. Further illustrating this principle, Figure 4H shows a case where, after having converged, perturbed spike trains diverged gradually across successive spike cycles ultimately resulting in separate populations of spike events (dashed box) riding on different fluctuations in the synaptic background (open red arrowheads). We categorized these phenomena as ‘divergence events’ and included all instances where perturbed spike trains for a given trial diverged after having drawn within 0.5 ms of the control spike train (mean across the 72 perturbations per trial).

Incidence of added spikes, skipped spikes, and divergence events across spike cycles—The incidence of added spikes was considerably higher with somatic stimulation than with distal dendritic stimulation, reflecting the relative strength of somatic perturbations. Added spikes were also more common during 15 Hz spiking (35.7% and 13.7% of trials for somatic and distal dendritic stimuli, respectively, across gain levels) than during 30 Hz (21.7% & 5.3%) or 45 Hz spiking (16% & 1%). The incidence of trials containing skipped spikes initiated by somatic inputs was very low across all synaptic backgrounds (0.6%) but somewhat larger for distal dendritic inputs (9.7%). These skipped spike events likely reflect hyperpolarization elicited by dendritic SK current evoked by excitatory stimulation. Consistent with this, in the GP_{NDSK} model, instances of skipped spikes were extremely rare (0.3% of trials) even with dendritic stimulation. There was no clear difference in the incidence of added spikes or skipped spikes between the mid-gain and high-gain synaptic backgrounds. However, both occurred much less often with the low-gain synaptic background. Divergence events were observed with a relatively low incidence for either somatic or distal dendritic stimuli (14.7% and 8% of trials, respectively) and did not exhibit a clear relationship with the gain of the synaptic background or with output spike frequency.

Stimulation of a single neuron can be sufficient to cause state transitions in local recurrent networks (Fujisawa et al., 2006), and individual added or skipped spikes have been shown to be capable of dramatically affecting global network activity in a large-scale thalamo-cortical model (Izhikevich and Edelman, 2008). To examine over what time period the dynamics of even a single cell can lead to sustained spike pattern disturbances we evaluated the incidence of added and skipped spikes, as well as divergence events, over an extended range of spike cycles subsequent to stimulation (Fig. 5A–C) and as a function of the phase of perturbing stimuli (Fig. 5D–F). Surprisingly, the occurrences of these event types over the 20 ISIs following somatic (Fig. 5A) or dendritic stimulation (Fig. 5B&C) did not strongly favor the first few ISIs, but were widely distributed over many spike cycles with a peak frequency occurring between 5 and 10 spike cycles subsequent to stimulation. This result highlights the power of individual synaptic inputs to influence the spike pattern of a postsynaptic neuron for many spike cycles even in the absence of network feedback.

Stimulus-phase-dependence of added spikes and divergence events—As illustrated in Figure 4E–H, spike addition, deletion and divergence events typically resulted from a sequence of interactions between the synaptic background at a given point in time and the recent spike history. Although the majority of added spikes and divergence events

did not take place at the time of stimulation, they did occur most commonly in trials where perturbing somatic (Fig. 5D) or dendritic stimuli (Fig. 5E) were delivered near the middle of the spike cycle. They were essentially absent, however, from trials with very early or late-phase stimuli which typically had a minimal effect on spike timing. These findings suggest that the phase of an input continues to be an important determinant of how a neuron will respond to synaptic fluctuations even after several spike cycles.

Stimulus-phase-dependence of skipped spikes—The incidence of skipped spikes showed the opposite pattern of stimulus-phase-dependence to that of added spikes. Skipped spikes were most likely to occur in trials where stimuli were delivered to the distal dendrite during a brief window early in phase or during the last 20% of the spike cycle (Fig. 5F). These are the same regions of the spike cycle during which distal dendritic stimuli tended to cause spike delays rather than spike advances (the negative regions in the F1 and F2 PRC; Fig. 3C2), suggesting a commonality in the underlying mechanism, i.e. activation of dendritic SK conductance. Consistent with this hypothesis, skipped spike events were almost never observed following dendritic stimulation of the GP_{NDSK} model.

Variability in spike time responses to perturbations is dependent on input phase—Coherent pallidal network activity may be capable of eliciting post-inhibitory rebound spiking in STN neurons (Hallworth and Bevan, 2005), and a feedback oscillation between STN and GP is a commonly proposed mechanism for synchronized oscillations among basal ganglia nuclei in Parkinson's disease (Plenz and Kitai, 1999, Magill et al., 2000, 2001, Terman et al., 2002, Loucif et al., 2005). However, in order for STN input to synchronize GP at the population level, the variance of the GP response to STN excitation and its phase dependence need to be considered. Therefore, we characterized the variability of shifts in spike timing elicited by our phasic excitatory PRC stimuli using phase response-variance curves (PRVCs).

Figure 6A illustrates that the variance in spike shifts elicited by somatic stimuli was maximal when stimuli were delivered during the middle of the spike cycle, and near zero when stimuli were delivered coincident with control spikes. Somatic PRVCs contained only a single peak in variance that diminished across spike cycles. Distal dendritic PRVCs (Fig. 6B), however, contained an additional peak in the F2 (blue arrowheads in B1, 2, & 3) for inputs delivered late in the spike cycle, corresponding to negative regions in the average PRCs shown in Figure 3C2&E2 and mirroring the incidence of skipped spikes shown in Figure 5E. This demonstrates that variability in the delaying effect of dendritic SK conductance is an additional source of variability in spike time shifts resulting from excitatory stimuli to the distal dendrite. The PRVC for distal dendritic inputs delivered against mid-gain synaptic backgrounds (1 nS unitary synaptic conductance) contained another additional peak for early-phase stimuli (Fig. 6B2, red arrowheads) which also corresponded to spiking delays seen in the average PRC (Fig. 3E2). This peak did not appear in the PRVC for the weaker (0.5 nS) synaptic backgrounds (B1&B3) indicating that, in this condition, the slight delays evoked in the first spike cycle were quite consistent (note the standard error of the corresponding average PRC in Fig. 3C2). The early-phase peak was also absent from PRVCs for the strongest (2 nS) synaptic backgrounds since in that case, delays of the first spike cycle were not robust to the highly fluctuating membrane conductance.

Elimination of the SK conductance from the dendrite (GP_{NDSK}) resulted in PRVCs for distal dendritic inputs (Fig. 6C) that did not contain multiple peaks and resembled the somatic PRVC for the GP_{base} model. The total variance in responses to dendritic inputs was greatly increased in the GP_{NDSK} model, indicating that dendritic SK can regularize spiking as we have previously described (Edgerton and Jaeger, 2011). Like the incidence of added and

skipped spikes, the variance in spike shifts evoked by somatic or dendritic stimuli generally followed the amplitude of the mean advances and delays illustrated by the average PRCs, but it is useful to consider that within a population of neurons receiving shared excitation, the subpopulation for which a shared excitatory input arrives at phases near a positive or negative peak in the PRC will also respond most variably to that input. Uncoupled populations of GP neurons would not be expected to synchronize to a greater degree than the variance shown in the PRC for any single cycle of rhythmic STN excitation, and heterogeneity among GP neurons might be expected to further diminish pallidal synchronization.

Decay of spike time perturbations depends on attractor, stimulus, and neuronal characteristics

Convergence depends on the gain of synaptic backgrounds, stimulus location, and dendritic SK conductance—We have previously demonstrated that physiological inputs to GP are not within the ‘weak-coupling regime’ which can be defined as the set of input strengths for which evoked shifts in spike timing scale linearly (Schultheiss et al., 2010). Furthermore, the effects of physiological inputs to GP were not limited to the stimulated spike cycle even in the absence of synaptic background activity. Therefore, to capture the full effect of phasic inputs on spike timing and synchronization, it is necessary to consider the duration (time) or number of spike cycles for which an input can be expected to influence the timing of subsequent spikes.

As described already, perturbations of control spike trains (spike time attractors) by PRC stimuli often persisted for many spike cycles, but ultimately the perturbations diminished and the control spike patterns were restored. The return of perturbed spike trains to the unperturbed trajectory can be measured using the Lyapunov exponent which reflects the time constant of exponential decay of the perturbation. For weak perturbations the Lyapunov exponent can be obtained from the infinitesimal PRC (Tateno and Robinson, 2007, Galan, 2009, Goldobin et al., 2010, Hata et al., 2011, Galan, 2012), however, in our simulations, PRC stimuli were physiologically ‘strong’ inputs, and nonlinear interactions with strong synaptic background fluctuations can ‘revitalize’ and prolong spike train perturbations. (Added and skipped spikes and divergence events illustrated in Figure 4 are extreme examples of this, although for the following analysis we excluded trials containing those events.) To summarize the decay of spike time attractor perturbations for different input locations and high conductance states, we defined ‘convergence’ as the progressive restoration of control spike patterns. We considered convergence to be complete when the 72 perturbed spike trains for a given trial, i.e. those in which a PRC stimulus was delivered, drew within 0.5 ms (on average) of the spike times of the respective control spike train. Then, to assess how the longevity of these perturbations depended on the synaptic background and on output spike frequency, we plotted for each synaptic background the proportion of trials that remained unconverged after each spike cycle following stimulus delivery (Fig. 7).

Convergence following somatic stimulation—Convergence following somatic perturbations progressed slowly across spike cycles for low gain synaptic backgrounds (nearly linearly, at least to a first approximation or by visual inspection) (Fig. 7A). Progressive convergence was also evident for the mid-gain and high-gain synaptic backgrounds, but the convergence was faster, reflecting the increased strength of higher-gain synaptic backgrounds (stronger spike time attractors) forcing the spiking trajectories back towards the control spike pattern. For example, approximately 40% of trials with low gain synaptic backgrounds remained unconverged after 10 spike cycles subsequent to perturbation, whereas only ~10% of trials with mid-gain synaptic backgrounds remained

unconverged after 10 spike cycles. There was a notable similarity in convergence across spike cycles following somatic stimulation independent of output spike frequency. Therefore, the number of spikes following the perturbation was more strongly related to the progress of convergence than was the time that had elapsed since the stimulus. This suggests that spikes themselves are a primary determinant in the restoration of the control spike pattern by dissipating the effects of the somatic perturbation. The mechanism underlying this effect likely consists of spikes being triggered by particular transients in the synaptic input pattern (spike time attractor), and when elicited, ‘resetting’ the membrane conductances by forcing the local voltage and membrane conductance activations through the relatively fixed trajectory of a spike.

Convergence following distal dendritic stimulation—Convergence following stimulation of the distal dendrite also exhibited a strong effect of synaptic background (attractor) strength (Fig. 7B). Perturbations of the distal dendrite generally persisted for fewer spike cycles than somatic perturbations, probably because of the smaller initial shift in spike timing. Since somatic spikes attenuate during backpropagation into the dendrites, the ‘resetting’ of dendritic membrane conductances (particularly SK) is likely much weaker than at the soma. Thus, in contrast to the pattern of convergence following somatic stimuli, the progression of convergence following dendritic perturbations showed a strong dependence on output spike frequency. When dendritic SK conductance was removed (GP_{NDSK} , Fig. 7C) the time course of convergence was qualitatively similar to the case of somatic stimulation.

Somatic and dendritic PRCs are robust against the elevated membrane conductance accompanying synaptic backgrounds—As described, larger voltage fluctuations accompanying higher-gain synaptic backgrounds caused responses to phasic PRC stimuli to be more variable across trials. After excluding trials in which added or skipped spikes or divergence events occurred, we obtained a ‘purified’ sample of trials with which to assess more directly the effect of synaptic background gain on the PRCs for somatic and dendritic stimulation (Fig. 8). The F1 somatic PRCs for higher-gain synaptic backgrounds were attenuated relative to the corresponding PRCs for weaker synaptic backgrounds (Fig. 8A); however, there was no significant difference in the amplitude (minima) of higher order PRCs for somatic inputs between the synaptic backgrounds of different strengths. These higher order PRCs were composed purely of negative values reflecting the diminution across spike cycles of the spike advances elicited in the stimulated spike cycle (Fig. 8A). The positive peaks of F1 PRCs for inputs to the distal dendrite were also attenuated by the elevated membrane conductance (reduced input resistance) particularly for the higher gain synaptic backgrounds (Fig. 8B) relative to PRCs derived during intrinsic spiking (Fig. 3B), but each still contained a slight negative region early in phase. Distal dendritic PRCs for the low-gain and mid-gain synaptic backgrounds also maintained a significant negative peak late in phase of the F2 PRC reflecting the delaying effect of SK current on the timing of the second spike following stimulation despite considerably elevated membrane conductance.

Upon close inspection of Figure 8, the F2 somatic PRC shows a negative peak at a similar phase to the positive peak in the F1 somatic PRC, whereas the F2 dendritic PRC contains a negative peak that is later in phase than the positive peak in the F1 dendritic PRC. This difference between somatic and dendritic responses (in terms of the symmetry of phase advances and delays across spike cycles) could strongly influence the consequences of excitatory inputs for network phase relationships including synchronization, but it is not readily apparent in the average across single-cycle PRCs. Furthermore, since synaptic backgrounds eventually restore the control spike pattern, the permanent PRC (calculated as the sum of F1, F2, and any additional higher-order PRCs) (Prinz et al., 2003) derived under

these conditions will nearly always be zero, indicating that stimulus effects on spike timing are eventually washed out. This is in contrast to phasic inputs to an intrinsically oscillating neuron which influence the timing of the next one or a few spikes, but after a few spike cycles a stable oscillation is re-established and the lasting phase shifts are captured in the permanent PRC.

Cumulative PRCs (cPRCs) distinguish the effects of intrinsic mechanisms from synaptic backgrounds

Cumulative PRCs (cPRCs) illustrate the evolution of input effects by assessing at each spike cycle the total shifts in spike timing (cumulative across spike cycles) relative to control traces evoked by inputs at each phase of the stimulated spike cycle (Fig. 9). Thus, the C1 PRC (C1 for the cPRC) equals the F1 PRC; $C2=F1+F2$; $C3=F1+F2+F3$; and so on. Average cumulative PRCs for the 'purified' sample of trials at 30 Hz are shown in Figure 9 for somatic and distal dendritic inputs to GP_{base} and for distal dendritic inputs to GP_{NDSK}. The somatic cPRC for GP_{base} was essentially composed of a single positive peak that attenuated smoothly over 10 spike cycles (Fig. 9A). The C1 PRC for distal dendritic inputs was also essentially a single positive peak; however, the C2 PRC contained significant negative regions both early and late in phase as a consequence of SK current evoked locally (Fig. 9B). Thus, while inputs delivered to the distal dendrite either early or late in the spike cycle had little effect on the timing of the next spike, they significantly delayed the second spike following the stimulus. The effects of the SK conductance on spike timing are captured by vertical translation of the C1 PRC to more negative values in the C2, which then attenuated across the next several spike cycles as the spiking pattern was driven back to the control trajectory by the synaptic background. Note, however, that the cPRC for distal dendritic inputs contains significant negative and positive peaks that persist for 6 to 7 spike cycles. These negative and positive regions in the higher order cPRCs indicate that for several spike cycles subsequent to stimulation, a subset of neurons in a population having received shared dendritic excitation will remain delayed relative to the control spike train while others will remain advanced, thereby restructuring the phase relationships within the network.

Phasic stimuli delivered to the distal dendrite of the GP_{NDSK} model yielded an average cPRC with much the same profile as the somatic cPRC for GP_{base} (Fig. 9C). Advances of the first spike cycle were reduced across successive spike cycles as illustrated by the attenuation of the positive peak. No delays were elicited by inputs at any point in phase, and convergence required 10 spike cycles (as with the somatic cPRC for GP_{base}) which was notably slower than for distal dendritic stimulation of GP_{base}. These results illustrate that dendritic SK conductance makes a fundamental contribution to the dendritic PRC over multiple spike cycles and results in sustained spike delays for inputs arriving either early or late in the spike cycle.

Discussion

The main goals of the present study were to determine whether and how synaptically driven high conductance states affect the phase response properties of GP neurons. The *in vivo* network activity can be seen as creating a spike time attractor to the individual GP neurons, and it is important to determine how phasic stimuli can change the time course of the attractor spike train and thus the embedding of the individual neuron into the network. These properties in turn will determine how the GP integrates into and shapes patterned or synchronous modes of basal ganglia network activity. We expanded the conventions of phase response analysis to assess the mean responses of the GP model to somatic and dendritic stimuli amidst synaptic background activity and to characterize the variability observed between trials resulting from the highly nonlinear interactions between synaptic background activity and phasic stimuli. For this we turned to computer simulations using a

morphologically reconstructed GP neuron model, because it gave us complete control over stimulus delivery to the dendrite and repeatability of spatially-distributed patterns of synaptic activity.

Mean phase response properties of the GP model

We found that for stochastic synaptic backgrounds of physiological amplitude, the pattern of shifts in spike timing evoked by PRC stimuli was well preserved compared to stimulation in the intrinsic pacemaking state, i.e. average somatic PRCs across trials were type I, and average distal dendritic PRCs were type II due to SK conductance activation. The pattern of frequency dependence for somatic and dendritic PRCs was also robust to the high conductance states tested, further indicating that the role of dendritic SK in promoting type II dendritic PRCs is not limited to intrinsic pacemaking. Our work here therefore suggests that the PRC paradigm can be generalized to high-conductance states when the average PRC over many stochastic input-driven trials is considered.

PRC stimuli in our simulations were physiologically realistic synaptic inputs, which exceed weak-coupling restrictions that are convenient for analytically predicting synchronization phenomena in networks. However, qualitative synchronous behavior in large coupled networks can be robust or heightened outside of the strictly defined domain of weak-coupling (Bogaard et al., 2009), and in our data the input phase dependence of spike time attractor perturbations illustrates that the intrinsic properties that underlie the PRC will continue to be important determinants of spike patterning and phase relationships in real neuronal systems which violate weak-coupling assumptions. Additionally, PRCs derived by our approach are likely to be effective predictors of other spiking phenomena which accompany fluctuating synaptic activity *in vivo*, such as added and skipped spike events whose incidence is also input phase dependent. Further PRC analyses in high conductance states to determine the single neuron contribution to synchronizing network properties are likely to provide a useful tool in general for studies of neuronal spike trains, oscillations, and phase relationships *in vivo*.

Stimulus interactions with synaptic fluctuations and phase dependent variance

Because the timing of synaptic background inputs were highly variable across trials, so too were shifts in spike timing evoked by PRC stimuli. We used PRVCs to directly measure the phase dependence of the variance with each synaptic background parameter set. This approach differs from previous methods of fitting the variance in experimentally measured PRCs (Netoff et al., 2005) as well as analytical methods developed to characterize the phase dependence of variance in the PRC which are mathematically accurate when noise and stimuli are relatively weak (Ermentrout et al., 2011). Compared to experiments, our approach benefits from the use of fixed patterns of synaptic background activity (similar to frozen noise) which allowed us to precisely determine the phase shifts of any spike in a spike pattern controlled by background inputs that is perturbed by a single PRC stimulus.

We found the variance in evoked shifts in spike timing to generally mirror the amplitude of the PRC in a phase and conductance-state dependent manner. For instance, the variance depicted in F2 dendritic PRVCs was very high at phases where the largest delays in spike timing appeared in the corresponding average PRCs. Despite the considerable differences of our input conditions from classic PRC theory in weakly coupled oscillators (Schwemmer and Lewis, 2012), these findings indicate that there is still a strong phase-dependence of input effects that is related to the original PRC shape.

Implications for pallidal network activity

The involvement of GP neurons in different modes of network activity in normal animals and disease models makes them a particularly interesting target for phase response analyses. In normal animals GP neurons are markedly desynchronized (Bar-Gad et al., 2003) perhaps in part as a consequence of perisomatically terminating inhibitory collaterals among GP neurons. Note, however, that such collaterals could also lead to synchronous spiking modes as shown in a recent network model of GP activity (Fujita et al., 2012). In animal models of Parkinson's disease (PD) in which GP single neuron activity has been recorded, GP neurons transition to a synchronous, rhythmic spike pattern (Nini et al., 1995, Raz et al., 2000), however, the observed synchronization does not consist of single spikes occurring at the same time.. Rather, it consists of a shared beta frequency modulated burst activity (Raz et al., 2000, Mallet et al., 2008). This transition potentially reflects increased susceptibility to entrainment to rhythmic excitatory inputs from the subthalamic nucleus (STN) (Magill et al., 2001, Mallet et al., 2008), and/or feedback activity within the reciprocally connected GP-STN microcircuit (Plenz and Kitai, 1999, Bevan et al., 2002, Terman et al., 2002). The underlying mechanisms for the emergence of rhythmic, synchronized activity within GP after degeneration of dopaminergic input to the basal ganglia likely involve a combination of cellular mechanisms such as a reduction of HCN channel density that reduces regular pacemaking in GP neurons and could enhance burst entrainment (Chan et al., 2011), and network factors such as an imbalance in the input arriving from striatum and STN to the GP (Holgado et al., 2010). Because most excitatory as well as striatal inhibitory input to GP (and most other types of neurons) is on dendritic sites (Shink and Smith, 1995), it is critical to consider the effects of dendritic inputs in the control of spike timing and network behavior.

Recent evidence indicates an orchestrating role for GP in the β -frequency synchronization of basal ganglia activity in PD (Mallet et al., 2008). Because an excitatory input from STN neurons would be expected to arrive at a population of GP neurons at different phases of their respective spike cycles, evoked shifts in spike times would vary across the population, restructuring the phase relationships among GP neurons. This is the conceptual basis by which the PRC can be used to predict patterning of network activity, and it is well established that type II PRCs like those that we describe for dendritic input to GP (Schultheiss et al., 2010) are predictive of synchronization phenomena (Galan et al., 2007a, Marella and Ermentrout, 2008, Abouzeid and Ermentrout, 2009, Bogaard et al., 2009).

Distal dendritic excitation of GP from STN is in principle well suited to be synchronizing via type II PRCs. However, β -frequency oscillatory activity in STN and GP that is a critical component of the pathophysiology of Parkinson's disease (Magill et al., 2001, Sharott et al., 2005, Mallet et al., 2008) is identified by a β -modulated bursty pattern of irregular spike trains, which are not entrained between neurons on a spike by spike basis. Indeed, when we subjected a version of our GP neuron model to input patterns based on STN spike trains recorded from 6-OHDA depleted rats (Mallet et al., 2008), we did not observe a specific role for dendritic SK currents in the entrainment of GP to such β -modulation in STN inputs (Edgerton and Jaeger, 2011). However, in these simulations there was no feedback connectivity and entrainment of network activity over many β -cycles remains to be determined in true network simulations. In this regard it is important to note that PRC analysis can also be carried out over burst cycles instead of single spikes, a type of analysis that has been explored in some detail in invertebrate preparations (Sieling et al., 2009).

Longevity of inputs' effects on spike timing as time windows of phase integration

As illustrated by the progression of convergence and particularly the cumulative PRCs, the effects of individual synaptic inputs can be long lasting despite considerable ongoing

synaptic background activity. This is a consequence both of the time-course of the intrinsic mechanisms activated by stimuli as well as the synaptic backgrounds. Note that the latter is a case particular to the high conductance state, because in the absence of ongoing synaptic background activity, stimuli would elicit permanent shifts in spiking phase. The strength of synaptic background activity constrains a neuron's 'memory' for previous individual inputs, i.e. the time window during which inputs' effects on spiking phase can integrate. Thus, it is important to interpret the effects of underlying intrinsic mechanisms on network organization across successive cycles of oscillatory input. However, in a large connected network it could be expected that the average PRC would dominate over specific cases where the spiking pattern is thrown off by a specific long lasting interference pattern caused by an individual input. We have interpreted the results primarily in the context of spike time attractors which entrain spiking to particular fluctuating input patterns. Stochastic synchronization whereby synchronous, albeit stochastic, spiking is driven in neurons receiving shared or correlated fluctuating inputs would not be expected based on our findings under normal GP conditions, because of limited input correlations. However, as we have described, it is possible that related mechanisms of synchronous activity could result in the Parkinsonian basal ganglia with heightened network synchrony within and between nuclei.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

We examined phase response curves in GP neurons during a high conductance state

Average PRCs with synaptic noise were close to those of an oscillator

Somatic input showed type I PRC and dendritic input showed type II PRC behavior

The variance of the PRC with synaptic noise was highest in mid spike cycle

Divergence effects such as added or skipped spikes were present in some trials

Figure 1. Diverse synaptic backgrounds achieve *in vivo*-like output spiking

A. Iso-frequency lines for GP_{base} . Each point on each line reflects a pair of excitatory and inhibitory input frequencies that generated output spiking at one of the target frequencies (indicated by color). Dashed and solid lines for each color trace reflect the unitary conductance (gain) of the synaptic events composing the synaptic background. **B.** Iso-frequency lines for the GP_{NDSK} model. **C.** Representative total excitatory and inhibitory conductance traces (top), total excitatory and inhibitory current traces (middle), and voltage traces (bottom) for synaptic backgrounds (indicated in **A**) with 1 nS unitary synaptic inputs (**C1**), 2 nS unitary synaptic inputs (**C2**), and high frequency inputs (**C3**) during 30 Hz spiking of the GP_{base} model. **D.** Representative traces for the GP_{NDSK} model with a synaptic background (indicated in **B**) driving 30 Hz spiking.

Figure 2. Synaptic background parameters determine inter-spike interval distributions
A. ISI histograms during control spiking for the 9 low input frequency parameter sets (indicated in Figure 1A). **B.** ISI histograms during control spiking for the 9 high input frequency parameter sets (also indicated in Figure 1A). **C.** ISI histograms during control spiking for the 9 parameter sets for the GP_{NDSK} model (indicated in Figure 1B). **D&E.** Coefficients of variation (CV) of ISIs for the GP_{base} model (**D**) and GP_{NDSK} model (**E**) during control spiking.

Figure 3. PRC shape is robust to fluctuating synaptic background activity

A. GP model morphology and stimulation sites. The model reproduces the morphology of a GP neuron recorded *in vitro*, filled with biocytin, and subsequently stained. Stimuli were delivered either to the soma (S) or a distal dendritic site (DD), i.e. the distal tip of the second dendritic branch which corresponds to D2_D in our previous publication (Schultheiss et al., 2010). **B.** Spike frequency dependence of somatic and dendritic PRCs when the model is driven by tonic somatic applied current. **B1.** Single cycle somatic PRCs are type I containing a positive peak in the F1. At higher spike frequencies the positive peak in the F1 PRC is increasingly opposed by a negative peak at the same phase in the F2. **B2.** Single cycle

dendritic PRCs are type II containing a negative early in phase (F1) for low spike frequencies which was shifted into higher order PRCs (F2) during faster spiking. **C.** Average somatic and distal dendritic PRCs across 100 trials with low gain (0.5 nS unitary conductance) synaptic backgrounds for spike frequencies spanning the *in vivo* range. Average somatic PRCs are type I (**C1**), whereas distal dendritic PRCs (**C2**) have multiple negative regions (green and blue arrowheads) that are more pronounced during slower spiking. **D.** Average somatic (**D1**) and dendritic PRCs (**D2**) during high input rate synaptic backgrounds. **E.** Average somatic (**E1**) and dendritic PRCs (**E2**) for mid-gain synaptic backgrounds (1 nS unitary conductance). As in **C**, green and blue arrowheads in **D2** and **E2** highlight negative regions in the PRC characteristic of the effect of dendritic SK. **F.** Average somatic (**F1**) and dendritic PRCs (**F2**) comparing the GP_{base} and GP_{NDSK} models. F1 somatic and dendritic PRCs are attenuated when dendritic SK is intact. The negative regions early in phase of the F1 and late in phase of the F2 dendritic PRC are eliminated when SK is removed from the dendrite (**F2**).

Figure 4. Individual somatic or dendritic synaptic inputs can lead to long-lasting perturbations of the spiking pattern

A. Voltage trajectories for a control simulation with a low-gain synaptic background (thick black line) and for 72 simulations each containing a single 2.5 nS synaptic input to the soma within the first spike cycle (color traces, color reflects the phase of the input as indicated by the color bar below the stimulated ISI). **B.** Voltage trajectories for single dendritic stimuli delivered during a low-gain synaptic background. (Note the blue traces showing delays of spiking.) **C&D.** Voltage trajectories for somatic (**C**) and dendritic stimuli (**D**) delivered during a high-gain synaptic background. **E.** Perturbations leading to an added spike. Spikes are numbered above each peak (in black for control spikes and red for spikes in simulations containing a stimulus). The open red arrowhead indicates a deviation of perturbed spiking trajectories from the control spiking pattern that initiates a sequence of events (dashed box)

leading to the added spike (solid red arrowhead). **F.** Perturbations leading to a skipped spike. The open blue arrowhead indicates a deviation of perturbed spiking trajectories from the control spiking pattern leading to the skipped spike (solid blue arrowhead). **G.** Perturbations leading to an added spike and subsequently, a skipped spike. These added and skipped spike events occur several spike cycles after stimulation and after the perturbed spiking patterns seemed to have converged back to the control spiking pattern. The red arrowhead indicates the initiation of a sequence of events that resulted in an added spike (first boxed region), and the blue arrowhead indicates a skipped spike that resulted after perturbed trajectories diverged again from the control spike pattern (second boxed region). **H.** Gradual divergence of perturbed trajectories over successive spike cycles (black arrows). The open red arrowheads indicate where perturbed trajectories spiked on a different set of fluctuations in the synaptic background than did the control simulation.

Figure 5. Incidence of added spikes, skipped spikes, and divergence events over successive spike cycles following stimulation and as a function of input phase

AB&C. Histograms of the number of added spikes, skipped spikes, and divergence events that occurred during the 20 spike cycles subsequent to stimulation. These types of events occurred during many spike cycles following stimulation of the soma of GP_{base} (**A**), the distal dendrite of GP_{base} (**B**), or the distal dendrite of the GP_{NDSK} model (**C**). **DE&F.** Histograms of the number of added spikes, skipped spikes, and divergence events as a function of the phase of the perturbing stimulus. Even though these events typically did not take place during the stimulated spike cycle (illustrated in **AB&C**), there was a strong dependence on the incidence of each type of event on the phase of the stimulus within the stimulated spike cycle.

Figure 6. Phase Response-Variance Curves (PRVCs); Variance of stimulus-evoked shifts in spike timing is phase dependent

A. PRVCs (for low-, mid-, and high-gain synaptic backgrounds in **A1**, **A2**, & **A3**, respectively) illustrating the variance of spike shifts evoked by somatic stimulation of GP_{base} contain a single peak that diminishes across spike cycles. Variance of shifts in spike timing was near zero when stimuli were delivered coincidentally with a spike in the control spike train (phases of 0 and 1). **B.** PRVCs for distal dendritic stimulation of GP_{base} are multimodal in the higher order intervals (F2–F5) reflecting multiple sources of variability. **C.** Like the PRVC for somatic stimulation of GP_{base} , the PRVC for distal dendritic stimulation of GP_{NDSK} contains only a single peak, indicating that dendritic SK conductance accounted for variance peaks early and late in the F2–F5 PRVC for dendritic stimulation of GP_{base} .

Figure 7. Convergence of perturbed spiking back to the control spike pattern takes many spike cycles

A. The proportion of trials that remained unconverged (having not drawn within 0.5 ms of spike times in the control spike train) over successive spike cycles for somatic stimuli with each synaptic background. Note that the progress of convergence was similar for synaptic backgrounds of the same gain (circled) independent of output spike frequency (indicated by color). **B.** Convergence following distal dendritic stimulation was faster than for somatic stimulation and was also dependent on the gain of the synaptic background. Note that convergence following distal dendritic perturbation also reflected a strong dependence on spike frequency (circles). **C.** Convergence following distal dendritic stimulation of the GP_{NDSK} model showed dramatically reduced dependence on spike frequency and resembled convergence following somatic stimulation of GP_{base} .

Figure 8. Gain of synaptic backgrounds only slightly affects average somatic and D2D PRCs
A. Average PRCs for somatic inputs to GP_{base} (after removal of trials containing added spikes, skipped spikes, and divergence events). **B.** Average PRCs for distal dendritic inputs to GP_{base} . The negative region late in phase of the F2 PRC is only noticeably reduced for the high gain case.

Figure 9. Cumulative PRCs make intrinsic and synaptic background contributions to shifts in spike timing distinguishable

A. Cumulative PRCs (cPRCs) for somatic stimulation of GP_{base} attenuate gradually over 10 spike cycles (**A1**) without a notable change in shape or y-translation (**A2**). Note, **A2** plots the same single cycle cPRCs (F1, F2, etc.) from **A1** on the same x-axis to allow direct comparison of shape. Changes in shape or y-translations of cPRCs reflect the contributions of intrinsic mechanisms to spike timing, whereas attenuation of cPRCs reflect the effect of synaptic backgrounds forcing the spiking trajectory back to the control pattern. **B.** Cumulative PRCs for distal dendritic stimulation of GP_{base} show significant positive and negative regions for each spike cycle after the stimulated cycle. These dendritic cPRCs attenuate over 6 to 7 spike cycles, notably faster than for somatic stimuli (**B1**), and show a pronounced negative y-translation between the F1 and F2 cPRCs reflecting the delaying effect of evoked dendritic SK on spike timing (**B2**). **C.** Like cPRCs for somatic stimulation of GP_{base} , cPRCs for distal dendritic stimulation of the GP_{NDSK} model show a single positive peak and attenuate over 10 spike cycles (**C1**) without a notable change in shape or y-translation (**C2**).