Stochastically Gating Ion Channels Enable Patterned Spike Firing through Activity-Dependent Modulation of Spike Probability

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Abstract

The transformation of synaptic input into patterns of spike output is a fundamental operation that is determined by the particular complement of ion channels that a neuron expresses. Although it is well established that individual ion channel proteins make stochastic transitions between conducting and non-conducting states, most models of synaptic integration are deterministic, and relatively little is known about the functional consequences of interactions between stochastically gating ion channels. Here, we show that a model of stellate neurons from layer II of the medial entorhinal cortex implemented with either stochastic or deterministically gating ion channels can reproduce the resting membrane properties of stellate neurons, but only the stochastic version of the model can fully account for perithreshold membrane potential fluctuations and clustered patterns of spike output that are recorded from stellate neurons during depolarized states. We demonstrate that the stochastic model implements an example of a general mechanism for patterning of neuronal output through activity-dependent changes in the probability of spike firing. Unlike deterministic mechanisms that generate spike patterns through slow changes in the state of model parameters, this general stochastic mechanism does not require retention of information beyond the duration of a single spike and its associated afterhyperpolarization. Instead, clustered patterns of spikes emerge in the stochastic model of stellate neurons as a result of a transient increase in firing probability driven by activation of HCN channels during recovery from the spike afterhyperpolarization. Using this model, we infer conditions in which stochastic ion channel gating may influence firing patterns in vivo and predict consequences of modifications of HCN channel function for in vivo firing patterns.

Introduction

Thermal fluctuations in the conformation of an ion channel protein can cause it to make spontaneous transitions between discrete conducting and non-conducting states [1,2]. Nevertheless, computational models of ionic conductances in a neuron generally assume the behavior of a population of ion channels to be deterministic and stochastic gating of ion channels is usually neglected in models of synaptic integration and spike initiation [3,4]. For a typical cortical principal neuron, this assumption can be justified by the very small amplitude of the conductance change and resulting membrane current caused by opening of a single ion channel compared to either the resting membrane conductance or the threshold current for firing of an action potential. However, when neurons are depolarized to near threshold, the threshold for initiation of action potentials, the biophysical mechanisms that underlie spike generation dictate that the effective membrane conductance becomes very low [5]. As a result, even small fluctuations in ionic current through relatively few ion channels could significantly alter the membrane potential and the initiation of action potentials [6,7]. Consistent with this possibility stochastic gating of membrane ion channels that determine the threshold for action potential initiation can influence the dynamic electrical properties of neurons [8–11]. However, little attention has been given to the consequences of stochastic ion channel gating for the patterns of spike output produced during active states in which the membrane potential is depolarized to near threshold.

We have focused on understanding the influence of stochastic ion channel gating on the integrative properties of stellate neurons from Layer II of the medial entorhinal cortex (MEC). These glutamatergic neurons provide cortical input to the hippocampal dentate gyrus [12,13]. Electrophysiological recordings reveal two unusual integrative properties of stellate neurons from the MEC [14–17]. First, during prolonged periods of excitation stellate neurons fire action potentials in stereotypical clustered patterns. The frequency of spikes within a cluster is approximately 8–14 Hz and is relatively independent of the average spike frequency, while the intervals between spike clusters are typically hundreds of milliseconds or longer [18]. The organization of clustered spike patterns appears to depend on a large and slow spike afterhyperpolarization (AHP) that is also independent of the overall...
Author Summary

Neurons use electrical impulses called action potentials to transmit signals from their cell body to their axon terminals, where the impulses trigger release of neurotransmitter. Initiation of an action potential is determined by the balance of currents through ion channels in a neuron’s membrane. Although it is well established that membrane ion channels randomly fluctuate between open and closed states, most models of action potentials account for the average current through these channels but not for the current fluctuations caused by this stochastic opening and closing. Here, we examine the consequences of stochastic ion channel gating for stellate neurons found in the entorhinal cortex. The intrinsic properties of these neurons cause characteristic clustered patterns of spiking. We find that in a model of a single stellate neuron that is constrained by previous experimental data clustered action potential patterns are produced only when the model accounts for the random opening and closing of individual ion channels. This stochastic model provides an example of a general mechanism for patterning of neuronal activity and may help to explain the patterns of spikes fired by entorhinal neurons that encode spatial location in behaving animals.

average spike frequency [18]. A second distinctive feature of stellate neurons is the emergence of prominent (~3–5 mV in amplitude) intrinsic membrane potential fluctuations upon membrane depolarization [14]. These fluctuations have been proposed to contribute to network rhythmicity due to their power in the theta frequency range (4–12 Hz), the prominent oscillatory patterns of spiking output generated by stellate neurons are produced in vivo [39]. Patterned Spiking from Stochastic Channel Gating

channels. Depending upon the cell type and even the subcellular compartment studied, \( I_h \) can lead to varied properties, from prevention of bistability [37] to regulation of dendritic spiking [38]. Therefore, understanding the properties of stellate neurons and their sensitivity to manipulations of \( I_h \) will likely require an account of the interactions between multiples classes of ion channels.

To better understand the impact of stochastic ion channel gating on the patterns of spike output from stellate neurons and to reconcile the contrasting views of the role of \( I_h \) in perithreshold oscillations and clustered patterns of spike firing, we addressed two questions. How do interactions of \( HCN \) channels with other membrane ion channels lead to the emergence of membrane potential oscillations and spike firing patterns recorded from entorhinal stellate cells? Could stochastic ion channel gating at potentials close to spike threshold influence the patterns of spike output generated by stellate neurons? We demonstrate that whereas a deterministic model of channel gating is sufficient to account for many of the properties of entorhinal stellate neurons at hyperpolarized membrane potentials, including the consequences of \( HCN1 \) deletion, a model with stochastically gating ion channels is necessary to reproduce the distinctive properties of stellate neurons near threshold. Examination of the model reveals that spike initiation is probabilistic and that the tendency to emit clustered spikes can be explained by a transient increase in the probability of spike initiation following recovery from the action potential AHP. We find that this transient increase in spike probability is primarily due to \( I_h \) and explains the role of \( HCN \) channels in the emergence of clustered patterns of spikes. Finally, we ask whether stochastic ion channel gating could contribute to patterns of spike output observed in vivo. We propose that stochastic gating of ion channels expressed by stellate neurons is crucial to their transformation of synaptic input into a patterned spiking output and places constraints on the development of models of entorhinal cortex function [39].

Results

To study the influence of stochastic gating of ion channels on the integrative properties of stellate neurons we implemented a single compartment model neuron endowed with ionic conductances derived from experimental data (see Materials and Methods). In the results sections that follow we first describe the key integrative properties of this model and show that they are similar to published experimental data. We then explore how clustered patterns of action potentials emerge in the model. Finally, to establish whether the model might explain firing patterns recorded from superficial entorhinal neurons in behaving animals, we simulate responses of the model to dynamic input.

Initially, we developed kinetic formalisms of the Hodgkin-Huxley type and solved for the resultant currents deterministically (Figure 1). Using the deterministic model we established that the single compartment model could account for the resting membrane properties of stellate neurons (Figure 1 and Table 1). As a further constraint we examined whether the model could account for previous experimental results in mice with global deletion of the gene encoding the \( HCN1 \) channel. Thus, in addition to a wild-type version of the model, we implemented a version in which the fast, large \( I_h \) was replaced by a smaller, slower current similar to that recorded in \( HCN1 \) knockout mice [18]. This single compartment, deterministic model replicated the basic effects of either \( HCN1 \) deletion or pharmacological blockade of \( I_h \) on the resting membrane properties of stellate cells (Figure 1 and Table 1).
While previous studies have investigated the consequences of stochastic Na$^+$ channel gating in models containing otherwise deterministic ion channels [21], as well as addition of a simulated stochastic Na$^+$ conductance during experimental recordings from stellate neurons [20], models of stellate neurons in which all of the ion channels are stochastically gating have not been explored. To

**Table 1. Passive membrane properties of the stellate neuron models.**

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type (Deterministic)</th>
<th>Wild-Type (Stochastic)</th>
<th>HCN1 Knockout (Deterministic)</th>
<th>HCN1 Knockout (Stochastic)</th>
</tr>
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<tbody>
<tr>
<td>$V_{\text{rest}}$ (mV)</td>
<td>$-63.15$</td>
<td>$-63.13 \pm 0.07$</td>
<td>$-75.01$</td>
<td>$-74.97 \pm 0.05$</td>
</tr>
<tr>
<td>$R_i^+$ (MΩ)</td>
<td>$33.5$</td>
<td>$34.2 \pm 1.1$</td>
<td>$61.7$</td>
<td>$59.3 \pm 1.7$</td>
</tr>
<tr>
<td>$R_i^-$ (MΩ)</td>
<td>$32.2$</td>
<td>$32.3 \pm 1.4$</td>
<td>$62.1$</td>
<td>$61.4 \pm 1.5$</td>
</tr>
<tr>
<td>$\tau_{\text{m}+}$ (ms)</td>
<td>$5.4$</td>
<td>$5.0 \pm 0.5$</td>
<td>$10.1$</td>
<td>$9.7 \pm 0.9$</td>
</tr>
<tr>
<td>$\tau_{\text{m}2}$ (ms)</td>
<td>$5.4$</td>
<td>$5.6 \pm 0.9$</td>
<td>$10.1$</td>
<td>$9.8 \pm 1.0$</td>
</tr>
<tr>
<td>Sag Ratio</td>
<td>$0.73$</td>
<td>$0.72 \pm 0.08$</td>
<td>$0.83$</td>
<td>$0.84 \pm 0.03$</td>
</tr>
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Input resistance ($R_i$) was defined as the ratio of the steady-state voltage change in response to positive (“+”) or negative (“−”) current injection from the resting membrane potential. Monoeponential fits to the initial voltage response were used to obtain the membrane time constant ($\tau_m$). The sag ratio is calculated as the ratio of the peak hyperpolarization divided by the steady-state hyperpolarization for the negative current injection. Parameter estimates from the stochastic models were determined from an average of 5 simulations. Errors are the standard deviation.

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examine the effects of stochastic channel gating, all channels in both models were converted to first-order Markov models [1,40,41]. Consistent with previous studies [6,8,9], we find that even with the substantial channel densities that are required to match current amplitudes to values from whole-cell recordings, channel noise can cause significant deviations from the mean current (Figure S1). Nevertheless, the average resting membrane properties of the model are unaffected by the presence of stochastically rather than deterministically gating ion channels (Figure 1 and Table 1).

Perthreshold Membrane Potential Fluctuations in the Stochastic Model

At membrane potentials just below the threshold for initiation of action potentials, stellate cells generate membrane potential fluctuations with a dominant frequency typically in the 5–10 Hz range [14,16]. Our previous experimental studies using HCN1 knockout mice indicate that, at any given membrane potential, HCN1 channels are not required for fluctuations in this frequency range, but rather HCN1 channels suppress low-frequency components of membrane potential activity [18]. However, the amplitude of the theta frequency fluctuations becomes larger with depolarization towards the spike threshold and if the absolute value of the membrane potential is not accounted for, then deletion of HCN1 channels can appear to reduce the amplitude of membrane potential fluctuations by lowering the most depolarized potential at which fluctuations can be maintained without triggering action potentials [18]. These results contradict proposed deterministic models for the generation of theta frequency fluctuations by stellate cells [17,26] and also suggest how failure to account for differences in membrane potential could lead to the conclusion that block of HCN channels abolishes theta frequency fluctuations [25]. Nevertheless, it has yet to be shown whether these experimental observations can be accounted for in a theoretical model.

We first examined the membrane potential of the stochastic models during injection of constant current of amplitude adjusted to the maximum possible without triggering action potentials (Figure 2). For the wild-type and knockout versions of the model this corresponded to respective mean membrane potentials of −51.6 and −53.4 mV. At these membrane potentials, the stochastic stellate neuron models show large fluctuations in membrane potential (~3–4 mV peak to peak; Figure 2), whereas the otherwise identical deterministic models show no fluctuations (Figure 2C). We found that the membrane potential fluctuations recorded over long epochs (20 s) are spectrally complex, but show peak activation between 3–10 Hz consistent with previous observations in stellate neurons in vitro [18,42]. Some previous studies have analyzed brief epochs in which the membrane potential fluctuations appear to be coherent oscillations [16,25,43]. Consistent with these studies, we also find that short epochs of membrane potential, recorded from simulations with the stochastic models, reveal clear autocorrelation peaks (Figure 2B) and dominant frequency components in the theta frequency range (Figure 2C and 2D).

Removal of the fast and large component of Ih in the knockout model resulted in an apparent shift in the peak of the spectral density to lower frequencies (~5 Hz) similar to previous experimental results in HCN1 knockout mice [18] (Figure 2C and 2D). By contrast, measurements made when controlling for membrane potential between the models, reveal that the knockout model has larger amplitude fluctuations ($V_{\text{avg}} = -53.7 \text{ mV}$, simulation time $t = 3 \text{ s}$; $\sigma_{\text{WT}} = 0.37 \text{ mV}$ $\sigma_{\text{KO}} = 0.47 \text{ mV}$; see also Figure S2), also consistent with experimental data [18]. In further agreement with previous experimental data [10], these effects can be explained by the ability of HCN channels to reduce the membrane impedance at low frequencies (Figure S2). As predicted by changes in impedance, responses to a white noise current stimulus, with standard deviation matched to the current noise recorded in the stochastic model, were enhanced in the deterministic knockout model compared with the equivalent wild-type model (Figure 2C). Phase plots of the relationship between membrane current and voltage during perithreshold fluctuations, revealed that Ih is a minor contributor (Figures S3) to the net membrane current changes that drive fluctuations. Thus, the stochastic model accounts well for the properties of subthreshold fluctuations and their dependence upon HCN1 channels reported previously [16,18,21,25,42]. This model is consistent with perithreshold fluctuations arising from interaction of stochastically gating ion channels other than HCN channels (Figure S4) [21], but with the amplitude and spectral properties of the fluctuations shaped by the presence of HCN channels and dependent on the average membrane potential at which the fluctuations are examined.

Ih Determines the Stability of the Perithreshold Membrane Potential

The most depolarized average membrane potential that can be maintained without initiation of an action potential appears to determine the maximal observable amplitude of membrane potential fluctuations and is altered both in the HCN1 knockout model (Figure 2) and in experimental recordings of stellate cells from HCN1 knockout mice [18]. To further assess the stability of the membrane potential prior to action potential initiation we injected slow, ramp-like currents that crossed spike threshold for both the wild-type (Figure 3A) and knockout (Figure 3B) versions of the model. We averaged the membrane potential from several sweeps in a time window 0.1–0.5 s before the initial action potential for each trial (Figure 3E). The spike-triggered averages (Figure 3D) revealed that removal of the HCN1-like current from the model causes spikes to initiate from a more hyperpolarized membrane potential (wild-type: −51.15+/−0.12 mV; knockout: −52.72+/−0.12 mV; $P = 4 \times 10^{-11}$; $N = 20$ total trials; Figure 3E). This difference between the wild-type and knockout models is independent of stochastic channel gating (Figure 3D), but is to be expected from the increased rate of depolarization resulting from the reduced membrane conductance following removal of HCN1 channels. However, for both of the deterministic models the membrane potential follows a more depolarized trajectory than in the corresponding stochastic models (Figure 3D). This is consistent with spontaneous membrane potential fluctuations in the stochastic models triggering action potentials relatively early during the ramp current. Consistent with the difference in responses to DC current injection (Figure 2), during the time-window preceding the spike, the more depolarized potentials in the wild-type model are associated with an increased standard deviation of the membrane potential due to stochastic channel gating (wild-type: 0.90+/−0.06 mV; knockout: 0.69+/−0.04 mV; $P = 0.003$; Figure 3E). The shift in membrane potential stability was accompanied by a small increase in the standard deviation of the time of the first action potential in the stochastic HCN1 knockout model (wild-type: 0.119±0.008 s; knockout: 0.152±0.015 s; Figure 3C; $P < 0.05$, $N = 60$ simulations), suggesting that HCN1 channels may increase the reliability of spike timing as well as the stability of the subthreshold membrane potential.
Clustered Patterns of Spiking Emerge When Models Contain Stochastically Gating Ion Channels

When stellate cells experience maintained depolarizing currents that drive action potential firing at mean frequencies less than 5 Hz, the pattern of firing is characterized by clusters of action potentials at a relatively high frequency (8–14 Hz) interspersed with silent periods [14,18,24]. We determined the conditions for initiation of spikes with mean frequencies less than 5 Hz, at which
clustered spike patterns might be expected. In the deterministic model the transition from silence to continuous action potential firing occurs when the amplitude of the injected current is increased above 258.4 pA and 320.5 pA for wild-type and knockout configurations, respectively. For the deterministic models this transition corresponds to a sharp transition from silence to repetitive spiking at ~6 Hz (wild type) and ~3 Hz (HCN1 knockout) and clustered spike patterns were not observed (Figure S5). By contrast, the current threshold for the transition between silent and spiking states was ~246 pA and ~308 pA for the stochastic versions of the wild-type and HCN1 knockout models, respectively. In both stochastic models, arbitrarily low firing frequencies could be obtained when the injected current was just above this threshold. When the mean frequency of action potentials was less than approximately 5 Hz, both stochastic models generated clustered patterns of spikes (Figure 4). Thus, stochastic ion channel gating enables clustered patterns of spikes to emerge during firing at low frequencies in response to input currents that are of insufficient amplitude to initiate action potentials in the corresponding deterministic model.

We next examined in detail the patterns of spiking that emerge when constant current injected into the stochastic model drives low-frequency action potential firing (Figure 4). Consistent with electrophysiological results [18,24,44], we find that the interspike interval (ISI) distribution of the stochastic model in response to constant current injection is multimodal, being characterized by both a dominant, short ISI mode as well as a wide distribution of long ISIs (Figure 4A). However, in the knock-out model this short latency peak is much broader than in the wild-type model (Figure 4A). Closer examination of the model behavior across a range of average firing frequencies revealed the characteristic tendency of stellate neurons to fire clustered action potentials (Figure 4B and 4D). The knockout version of the model reveals a lesser tendency to fire spikes in clusters (Figure 4C and 4D),
Figure 4. Clustered spiking in the stochastic model. (A) Examples of interspike interval histograms calculated from long duration simulations (150 s) of the response of the wild-type (WT; left) and knock-out (KO; right) models to DC current injection. In both examples the mean firing rate is in the 1–2 Hz range. ISI distributions were fit with multiple Gaussians (solid blue lines). Insets show individual peak fits for the 0–0.6 s interval of the histogram. (B–C) Examples of 10 s duration epochs of membrane potential activity from simulations with the wild-type (B) and knockout (C) models. Average firing rate for the trial is stated in blue. (D) $P_c$ is plotted as a function of average firing rate for the wild-type (closed symbols) and knockout (open symbols) models using the ‘stringent’ clustering definition (left panel) and the ‘relaxed’ clustering definition (right panel). Several hundred, 16 s duration simulations of the partially stochastic model (Figure S6) were used to provide detailed sampling. (E) Number of spikes per cluster is plotted for a subset of the data.

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consistent with the broadening of the short latency peak in the ISI histogram (Figure 4A). We quantified the probability of clustering (Pc) with definitions used previously for experimental data (see Materials and Methods; [18]). For the wild-type stellate neuron model Pc depends upon average firing frequency and peaks at intermediate (1–5 Hz) frequencies (Figure 4D). Importantly, Pc is significantly reduced in the knockout model at intermediate average firing rates (Figure 4D). Finally, as in experimental recordings, the average number of spikes per cluster in the stochastic models is quite variable and depends on the average firing frequency (Figure 4E).

**HCN Channels Influence the AHP Waveform in Stochastic and Deterministic Models**

We previously demonstrated that I\(_h\) accelerates the repolarization from the AHP in stellate neurons, while overall shorter AHPs predict an increased tendency of neurons to fire clustered patterns of action potentials [18]. Similarly, the half-width of the AHP in the wild-type stochastic model was independent of the average frequency of spike firing (Figure 5C). In contrast, after the simulated removal of HCN1 channels, the AHP half-duration was broader and varied as a function of average spike frequency (Figure 5C), just as in experimental recordings from stellate neurons in HCN1 knockout mice [18]. The increase in duration of the AHP following removal of HCN1 channels was found in both stochastic and deterministic (Figure S5) versions of the model indicating that this role of I\(_h\) does not require stochastic gating of the membrane ion channels. To quantify spike initiation following the AHP we calculated the conditional probability that a spike occurred at a time \(t\) following a previous spike at time \(t_0\). For spike trains generated by the knockout model (Figure 5B, right panels), the latency to the increase in P\(_{st|st0}\) following a spike was increased and the magnitude of the change in P\(_{st|st0}\) was reduced from more than 6 fold to less than 3 fold compared with spike trains generated by the wild-type model (Figure 5A, right panel). These changes are correlated with the reduction in \(P_c\) observed in simulations of the knockout model across a range of firing frequencies (Figure 4D).

Together, these simulations indicate that deterministic or stochastic versions of our model stellate neuron are sufficient to account for the resting membrane properties, subthreshold stability of the membrane potential and the sensitivity of these properties to alteration of I\(_h\). However, only the version of our model containing stochastically gating ion channels is able to further account for the spontaneous emergence of membrane potential fluctuations at potentials near threshold. Moreover, the stochastic models produce clustered patterns of action potentials similar to spike patterns recorded from stellate neurons from wild-type and HCN1 knockout mice. Since our characterization of the stochastic models suggest that they provide a remarkably good account of experimental observations of both the resting and active properties of entorhinal stellate neurons, we went on to use these models to investigate how stochastic ion channel gating influences spike initiation and the generation of distinctive clustered patterns of action potentials.

**Clustered Firing Patterns Involve Brief Action Potential Dependent Changes in Firing Probability**

How do the clustered patterns of action potentials emerge and why do they require stochastic ion channel gating? In a deterministic neuron, clusters or bursts of action potentials arise through modulation of spiking by slow changes in the state of one or more ion channels [46,47]. Indeed, such a deterministic mechanism has previously been proposed to account for clustered patterns of action potentials fired by entorhinal stellate neurons [26]. By this account, stochastic ion channel gating may lower the threshold for spike generation, but is not essential for the generation of clustered patterns of activity. However, stochastic ion channel gating may permit mechanisms for control of spike patterns that are not possible in deterministic models. In particular, whereas initiation of an action potential in a deterministic neuron is binary, with a clearly defined threshold, for stochastic neurons fluctuations in ion channel activity can lead to cancellation of a spike even when the deterministic threshold is crossed. At the other extreme spikes can be initiated in conditions that are well below the deterministic spike threshold [8,9]. Therefore, in a stochastic neuron there is no clearly defined boundary between a spiking and a non-spiking state and thus spike initiation should be considered probabilistic rather than binary.

The probabilistic nature of spiking in the stochastic model leads to a simple alternative mechanism for generation of clustered patterns of spikes, whereby the transient elevation in the probability of spiking following a previous action potential is sufficient to produce patterned output (Figure 6). According to this mechanism, changes in the recovery from a spike would alter the pattern of spikes by modifying the spike probability immediately following the refractory period (Figure 6B). As a result, the activation of ion channels during each action potential and its associated AHP can be independent of the position of the action potential within or outside a cluster. Several lines of evidence support this probabilistic mechanism.

First, conditional probability distributions, P\(_{st|st0}\) (Figure 5A and 5B, right panels; also see Materials and Methods), reveal that the wild-type version of the model produces clustered action potentials by elevating the conditional probability of firing a spike, P\(_{st|st0}\), over the steady-state probability, P\(_{st}\), for a brief period of ~50 ms following a spike (Figure 5A, right panels). Moreover, the reduction in \(P_c\) in the HCN1 knockout model is correlated with a decrease in P\(_{st|st0}\) (Figure 5B, right panels) as required by a probabilistic mechanism for clustered firing (Figure 6B).

Second, the number of spikes within a cluster is variable for a particular firing rate (e.g. 3.11±1.7 spikes per cluster for 1.6 Hz) and depends upon the average firing rate in both our model (Figure 4E) and experimental data [18]. This suggests that the number of spikes in a cluster is probabilistic and is consistent with a stochastic model of spike generation, but distinct from previous deterministic models [26].

Third, in a deterministic mechanism the half-width of the AHP should systematically vary with position in the cluster and should determine the succeeding ISI when terminating a cluster. Thus, on a spike-by-spike basis we would expect the AHP to correlate with the subsequent ISI. However, we find no such correlation in spike trains from either the wild-type or knockout models (Figure 5D). Nonetheless, in both population data from experiments and in different versions of the stochastic model the AHP half-width correlates with \(P_c\). These observations therefore support our conceptual model of spike patterning and suggest that there may be a common ionic basis that regulates the time course of both the AHP and P\(_{st|st0}\).

Fourth, to generate activity patterns that take place over relatively long time scales, such as spike clusters, a deterministic model requires relatively slow changes in the state of the model and at least one of the model parameters must vary as a function of a spike’s location within a cluster. By contrast, the probabilistic mechanism of spike clustering does not require slow changes in model parameters beyond the recovery period from the AHP.
Figure 5. Recovery from the AHP is influenced by Ih and reflects spike clustering. (A–B) Overlaid action potentials (left) and corresponding conditional spike probabilities (right, truncated at $P = 0.1$) from long simulations (150 s) in which constant current was injected to the wild-type (A) and knockout (B) models. Average firing rate from the selected trials is indicated in blue. (C) Average width of the AHP at $-52 \text{ mV}$ for the wild-type (closed circles) and knockout (open circles) spikes. (D) For a representative trial (upper panels in A and B) a log-log plot of the AHP width against the succeeding ISI for wild-type (closed circles) and HCN1 knockout (open circles) versions of the model.

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(Figure 6). Consistent with this prediction we find that the distribution of currents during AHP recovery is not different between the first spike in a cluster and all other spikes regardless of their position (see below, Figures 7 and S8).

Fifth, the conditional spike probabilities ($P(s_i|s_0)$) are sufficient to generate spike trains with interspike interval histograms and clustered patterns of spikes that are indistinguishable from spike trains generated by the biophysical neuronal models (Figure 6B).
and 6C). Thus, \( P(s_t | s_{t0}) \) can fully characterize the spike train. By contrast, if there were higher-order correlations in the spike probabilities, as would be the case in any deterministic model of clustered spiking, then the conditional probabilities would differ for each spike and no single set of conditional spike probabilities would fully characterize the spike train [45].

**Transient Increases in Spike Probability Following AHPs Are Associated with an Inward Shift in the Balance of Membrane Currents**

In principle, the transient increase in the probability of action potential firing that occurs following recovery from the AHP could arise through a number of mechanisms: (1) A transient shift in the balance of membrane currents that together determine the overall direction and rate of change of the membrane potential; (2) A change in the stochastic current fluctuations that act as the noise source that enables probabilistic spike firing; or (3) A reduction in the threshold for spike initiation.

To address the first possibility, we evaluated the membrane current at a narrow range of membrane potentials (250.5 to 249.5 mV), just below the voltage threshold for spike initiation (Figure S8). In the stochastic model the membrane potential enters this range during silent epochs when spikes are not initiated, immediately before initiation of the first spike in a cluster and in the epoch following recovery from the AHP when a subsequent spike may or may not be triggered. We therefore assigned each membrane potential measurement to one of three different classes (Figure 7A): a 50 ms window prior to spike initiation from steady state (red); during AHP recovery of all spikes without regard to their position within a cluster (blue); and silent epochs during which no spiking occurred during or in the subsequent 100 ms (black). For each point within these time windows we sampled the...
membrane current if the membrane voltage was within the range −49.5 to −50.5 mV and then generated histograms of the membrane current for each epoch. Comparison of these three cases revealed that spike initiation from steady state is associated with a small but significant inward shift in the net ionic current relative to periods of silence (Figure 7A). The small shift in the mean current is consistent with the low average firing frequency (i.e., low P(st)). By contrast, the recovery from the AHP is associated with a larger shift (≈8 pA) of the net membrane current in the inward direction (Figure 7A), consistent with the increase in P(st|st0) relative to P(st) following AHP recovery and with the shift observed for spikes that initiate clusters (Figure S8). Thus, during the period following recovery of the AHP, the membrane experiences on balance a greater net inward current at potentials approaching threshold, driving further depolarization of the membrane potential and spiking.

We also evaluated whether other mechanisms might contribute to the change in firing probability following recovery from the AHP. Importantly, we found no difference in the standard deviation (σ) of the membrane current prior to initiation of spikes from steady state (σ = 16.6 pA), compared with AHP recovery (σ = 16.6 pA) or silence (σ = 16.5 pA), indicating that stochastic current fluctuations have a similar magnitude in each condition (Figure 7A). Moreover, there was no correlation between the membrane potential at which we detected spike initiation (see Materials and Methods) and the preceding ISI for either the wild-type or knockout models (R² = 1.10⁻⁴; Figure S7), indicating that the brief elevation in P(st|st0) is not due to an alteration in the voltage threshold following a previous spike. Thus, the shift in average membrane current, as opposed to a change in the stochastic current fluctuations or spike threshold, appears to be the major determinant of increased firing probability following the AHP.

Figure 7. Ih during AHP recovery enhances spike probability and clustering. (A) Probability density plots for the magnitude of the net ionic current within the voltage range −49.5 to −50.5 mV taken from epochs in which no action potentials occurred (“Silent”; black), preceding the initial spike of a cluster (“Initial Spike”; red), or, during recovery from the spike AHP (“AHP”; blue). Each plot is fit with a Gaussian function, which was used to estimate the standard deviation of the distribution. Areas were normalized to P = 1 and all distributions had nearly identical properties (σ_{steady-state} = 16.6 pA, σ_{AHP} = 16.6 pA, σ_{silence} = 16.5 pA). (B–C) Probability density plot for Ih (B) and for INaP (C) during the same simulation epoch as in A. (D) Color-coded plot of the average membrane potential for all action potentials. Transition from red to blue color applies to E and F. Solid lines are derived from fits of Gaussian functions. (E) Phase plot of the mean Ih during the spike. (F) Phase plot of the mean INaP during the spike. (E–F) Insets focus in on the region of membrane potential selected for the plots in A–C.

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Slow Activation and Deactivation of Ih Determines the Time Window for Increased Spike Probability Following an AHP

Since our experimental and modeling data indicates that HCN channels influence both the AHP and clustered spiking, we asked whether changes in Ih during the AHP could account for the shift in membrane current that underlies the increase in $P(st|st_0)$ relative to $P(st)$. Importantly, the shift can be fully explained by an increase in the amplitude of Ih during AHP recovery (Figure 7B). By comparison another current important for spike initiation, the persistent sodium current ($I_{NaP}$) shows no change (Figure 7C). Consistent with this explanation, phase plots for $I_{NaP}$ (Figure 7E) and $I_{NaP}$ (Figure 7F) during an action potential, reveal an increased Ih density associated with recovery from the AHP.

Are the kinetics of Ih important for the relatively brief increase in $P(st|st_0)$ that appears to underlie generation of clustered patterns of activity (Figures 5 and 6)? Simulated voltage-clamp of isolated Ih using a command potential based upon the action potential waveform (Figure 8), revealed an increased density of Ih following recovery from the AHP (Figure 8B). Comparison of the observed Ih ($I_{h,obs}$) with the current density predicted from the steady-state I–V relationship for Ih ($I_{h,s}$), revealed that while $I_{h,obs}$ was less than $I_{h,s}$ at time points corresponding with the peak of the AHP, during the return phase of the AHP $I_{h,obs}$ is larger than $I_{h,s}$ (Figure 8C and 8D). This transient elevation in Ih relative to steady-state precedes the time course of $P(st|st_0)$ with an expected lag for action potential initiation and detection (Figure 8D). To determine if this shift in the net membrane current could cause the shift in firing probability, we simulated an increase in the injected current by the peak value of $I_{h,obs} - I_{h,s}$. The increase in $P(st)$ (dashed red line; Figure 8D) during this simulation relative to $P(st)$ under the control simulation (dashed blue line; Figure 8D) accurately predicts the peak of $P(st|st_0)$. Thus, a brief change in the net inward current due to Ih during the AHP appears to be sufficient to explain the magnitude and time course of $P(st|st_0)$.

The Slow Gating Kinetics of Ih Are Important for Clustered Spiking in the Model

To directly test the influence of the slow gating kinetics of Ih on action potential clustering we scaled the forward and reverse rates of the closed-open transition of Ih (Figure S9). While the kinetics did not alter the magnitude of the steady-state current, it did allow Ih to equilibrate to the membrane potential during recovery from the AHP (Figures 8E and S9) and significantly reduced the short-latency (~100 ms) peak in $P(st|st_0)$ (Figures 8F and S9). This reduction in spike probability following a prior spike resulted in a 33% reduction in $P_c$ for a 1–2 Hz average firing rate. However, changing the kinetics of Ih complicates this analysis and likely leads to an underestimate of the effect. For example, the change in kinetics leads to a 10% reduction in the AHP half-width and increases the stochastic fluctuations in Ih about its mean, both of which effects could increase $P_c$. Stochastic gating of HCN currents is not necessary for clustered spiking (Figure S6). Thus, we also ran simulations with fast, deterministic HCN channels to prevent the increase in fluctuations and found that $P_c$ was reduced 40% to 0.33, close to the theoretical minimum of 0.29 for a refractory Poisson process where $P(st|st_0)$ is equal to $P(st)$ (Figure 6).

Together, these data suggest that activation of Ih during the AHP is an important determinant of both the AHP half-width and the clustering of action potentials. Given the relatively slow kinetics of Ih, the closing of HCN channels lags the depolarization of the membrane on the tail of the AHP and Ih fails to equilibrate to the membrane potential. As a result, the AHP recovery is associated with a transient increase in Ih relative to steady-state that contributes to an increase in the probability of action potential initiation. Moreover, this effect is robust across a range of channel kinetics tested (Figure S9). However, due to their relatively small single channel conductance [46], changes in mean HCN current act primarily as a DC bias current, rather than as a noise source.

The Stochastic Model Can Account for Firing Properties of MEC Neurons In Vivo

Could the stochastic model that we outline here also explain aspects of the firing patterns of neurons in behaving animals? Consistent with this possibility, spike times obtained from in vivo single unit recordings [49] show elevations (made clear by exponential bin spacing [50]) in their ISI distribution at around 10 ms (Figure 9E). This ISI resembles the peak of $P(st|st_0)$ in simulations of our stochastic model, but unlike the responses of our model to constant current input, the in vivo spike trains contain a much broader overall distribution of ISIs. To provide a more realistic comparison between the model and in vivo data, we therefore carried out simulations of the response of the model neuron to simulated synaptic drive.

To reduce the uncertainty of comparing the model output with in vivo recordings during which the physiologically relevant inputs are unknown, we first examined a wide region of stimulus space by varying the standard deviation and offset of a band-limited, white noise stimulus ($f_{max} = 50$ Hz). In this way, we obtained a description of the relationship between properties of the simulated input to the model and the mean frequencies (Figure 9A) and coefficient of variation (CV; Figure 9B) of the ISI distributions generated by the spike outputs from the model. Based on comparison of these data with the frequency and CV of spike trains recorded in vivo (Figure 9C), we selected for use in further simulations parameters that generated spike trains with CV and ISI spanning the space covered by the in vivo spike data (Figure 9D). For inputs with a large standard deviation and a small offset there were only small differences between output responses of the stochastic and deterministic versions of the wild-type model (Figure 9F; $\chi^2$-test, $P = 0.01$). In contrast, for inputs with a large offset and small standard deviation, striking differences were apparent between the responses of the stochastic and deterministic models (Figure 9G; $\chi^2$-test, $P < 0.0001$). In both cases the stochastic model tends to redistribute the average ISI distribution such that it is enriched for 100–200 ms ISIs, but this effect is greater for the responses to weakly varying inputs (Figure 9H).

Unlike the in vivo experimental data, the simulations above did not generate high frequency (>25 Hz) bursts of spikes. However, examination of the stimulus space indicated that high variance stimuli with substantial DC offsets could produce spikes at high frequency (Figure 9A). Since recordings of the local field potential in the medial temporal lobe in vivo indicate that the network is characterized by long periods of relatively uncorrelated activity interspersed with brief epochs of highly correlated activity [51], we attempted to mimic these stimulus statistics by assuming that the stimulus can be characterized by a relatively low average variance (characteristic of uncorrelated presynaptic activity) interspersed at random (Poisson) delays ($\lambda = 1$ s) with random duration ($\lambda = 200$ ms) epochs of high average variance (characteristic of correlated presynaptic activity). This pattern of stimulation is illustrated graphically as a transition between two points in stimulus space (Figure 9D) and resulted in a much broader ISI distribution that more closely matched the in vivo data (Figure 9I). Under these stimulus conditions, simulations of the stochastic model also resulted in an ISI histogram enriched for intervals
Figure 8. Elevated $I_h$ during AHP recovery correlates with increased spike probability. (A) The voltage command ($V_c$) waveform used for voltage-clamp simulations (left). The voltage command in the region indicated by the box is also shown on an expanded scale (right). Horizontal line indicates initial value of the command potential. Vertical line indicates time at which command returns to its initial value. (B) Isolated $I_h$ during voltage-clamp of the model to the command potential in A (average of 10 simulations). (B, right) Isolated $I_h$ during voltage command return to steady state. The plot corresponds to the region of the voltage command highlighted in the right hand panel of A. Solid black line indicates average of 10 simulations shown individually in gray. Vertical and horizontal lines as in A. (C) Observed $I_h$ (red) is plotted along with the steady-state $I_h$ density expected at each potential in the command waveform. (D) Plot of the difference between the observed and expected steady-state $I_h$ ($I_{\text{obs}} - I_{\text{ss}}$) during the period of AHP recovery in the command potential. Superimposed is the plot of the probability of an action potential, $P(s|s_{\text{st}})$, for the clustering
The Patterns of Spike Output from Neurons with Stochastically Gating Ion Channels Can Be Controlled by Activity Dependent Changes in Spike Probability

The rules that determine transformation of synaptic input into patterns of spike output are fundamental to computations carried out within the central nervous system. While models of cortical neurons take advantage of simplifying assumptions that characterize spike output as an invariant function of synaptic input (e.g., [35, 34]), experimental recordings suggest that stellate neurons from layer II of the MEC generate clustered patterns of spike output through intrinsic mechanisms that may not be reducible in this way. In the biophysical model of a stellate neuron that we develop here, a brief increase in spike probability immediately following recovery from a preceding action potential can substantially modify the pattern of spike output. In the low firing frequency regime, spikes can be initiated by random fluctuations of the net membrane current. As a result of the balance of currents near threshold, the low effective membrane conductance, and the relatively large currents that can be produced by individual ion channels, small bias currents can substantially alter the probability of firing by shifting the mean of the net membrane current. This model is sufficient to explain the clustered patterns of spikes that are recorded from stellate cells during injection of constant current (Figure 6). This mechanistic account also provides some suggestion that the tendency of neurons in layer II of the MEC to fire spikes at 5–10 Hz may result from the transient, spike-dependent increase in spike probability that can influence spiking even in the presence of a continuously varying barrage of synaptic inputs (Figure 9).

The model that we develop here differs from a number of other models proposed to explain the integrative properties of stellate neurons. Two previous, biophysically-detailed deterministic models have proposed that cyclic interactions between $I_{NaP}$ and $I_h$ are necessary and sufficient to produce perithreshold oscillations [25, 26]. However, this conclusion is not supported by experimental observations from stellate neurons following genetic deletion of HCN1 [18], or pharmacological block of $I_h$ [22]. One of these previous biophysical models also produces patterned spiking, although quantitative comparisons of the patterns produced with experimental data have not been reported [26]. This previous model requires slow deterministic changes in model parameters to produce clustered patterns of spiking, whereas the model we propose here demonstrates that such slow changes are not necessary for the emergence of clustered spike firing. Nevertheless, it is possible that in entorhinal stellate neurons slow changes in ion channel states could further influence spike firing patterns in addition to the activity-dependent changes in spike probability that we describe here.

A conductance-based stochastic model [21] and a more abstract stochastic resonate-and-fire (SIF) model [44] have also been developed to account for the properties of stellate neurons. These models successfully account for the complex spectral properties of perithreshold fluctuations of membrane potential that are recorded experimentally and that are also generated by the stochastic model we describe here. It was previously suggested that a simplified, stochastic $I_{NaP}$ is sufficient to produce patterned
Figure 9. Effects of stochastic channel gating alter the response to stellate cells to naturalistic stimuli. (A,B) Plot of mean spike frequency (A) and coefficient of variation of the ISI distribution (B) as a function of the mean and standard deviation of band limited white noise inputs obtained from 5 s duration simulations (N = 384). (C) CV plotted as a function of mean firing frequency for the same data shown in A and B. The frequency and CV of several recordings (see [49]) from neurons in the superficial layers of the medial entorhinal cortex in vivo are plotted for comparison (red dots). These values from in vivo data were used to define a region of stimulus space selected for further analysis (red box). (D) A masked plot of stimulus space shows the simulations that resulted in values within the red box defined in C. Longer simulations (150 s) were run for the points indicated in red using both the deterministic and stochastic models. (E) The mean ISI probability density for experimental recordings plotted in C. Gray shaded region indicates the range of ISIs for spike clusters (see text). (F, G) ISI histograms obtained from simulations with the
deterministic ("D") and stochastic ("S") versions of the model using input statistics at the extrema of the plot in D (indicated by "F" and "G"). (H) The difference in spike counts between the D and S simulations for the data plotted in F (blue) and G (red). The stochastic model shows a selective redistribution in the probability of spiking that produces an increase in the clustering interval (shaded region) and a decrease at longer ISI intervals. (I) ISI histograms obtained from simulations with deterministic (blue) and stochastic (black) versions of the model using input statistics that fluctuate randomly between the two states indicated by the double-headed arrow in D. (J) The difference in spike counts between the D and S simulations for the data plotted in I. (K) ISI histogram for response of the knockout model to the unscaled ("us"; blue) and the scaled ("s"; red) poisson stimuli (see text). Gray shaded region is the data from I replotted. (L) The difference in count between the "us" and "s" simulations for the data plotted in K. All histograms use exponentially spaced bins.

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spiking [21] through random threshold crossings and spike omissions [8,9]. However, the spiking patterns produced due to a stochastic I_{NAP} alone are nearly identical to a stochastic point process with a refractory period and thus do not provide a good match to the patterned observed experimentally [see Figure 4 in [21]]. By contrast, the stochastic model we describe here produces more complex spike patterning that is a better match to the characteristics of clustered firing observed experimentally and not well described by a refractory Poisson process (Figure 6). The mechanism that we suggest for generation of clustered firing patterns also differs markedly from a recently proposed resonate-and-fire model that also reproduces clustered firing patterns of stellate cells [44]. The resonate and fire model explicitly states that sub-threshold resonance mechanisms are required to generate clustered spike patterns, whereas recent experimental studies clearly dissociate sub-threshold resonance from clustered spike firing patterns of stellate cells [18,24]. Consistent with this data, the probabilistic model that we propose does not require sub-threshold resonance for generation of clustered spike firing and can provide a mechanistic explanation for dissociation of these two properties.

Limitations of the Stochastic Model

There remain features of the firing patterns recorded experimentally from stellate neurons that are not well captured by any model proposed so far. A striking feature of some stellate neurons is a fairly regular intercluster interval even in the absence of coherent subthreshold oscillations (e.g. Figure S10). Our model stellate neuron, however, appears to exhibit more widely distributed intercluster intervals. One likely cause of this discrepancy is the simplification of the AHP current used in the model. Indeed, early results demonstrated that blockade of calcium entry can reduce the tendency of spiking to be clustered [15]. Since neuronal morphology can influence patterns of spike output [55], a further important limitation to the model that we propose here is that it is composed of only a single compartment. On the one hand, this could lead to an underestimate of the influence of stochastic gating, as in an extended dendritic structure fewer ion channels would contribute to ionic currents in any single compartment and thus the influence of stochastic channel gating on the membrane potential would be greater, as has been argued to be the case for thin axons [56]. On the other hand, comparison between our simulations and experimental data suggest that the magnitude of perithreshold oscillations and extent of spike clustering are comparable or perhaps larger in the model. Assessing the contribution of stochastic ion channel gating to the spatially distributed properties of stellate neurons will require future studies with more detailed computational models developed in parallel with more detailed electrical measurements from spatially distinct regions of the neuron. Nonetheless, the general principles that we establish here are likely to be robust to differences in morphology and although further morphological data may improve the similarity between our model data and the experimental data, simple models of neurons, neural circuits, and behavior can provide important functional insights in the absence of exhaustive detail [57].

HCN Channels Support Patterned Spiking

Analysis of the stochastic model supports a key role for HCN channels in controlling the pattern of spike output from stellate neurons and suggests how the unique biophysical properties of HCN channels enable this role to be achieved. Thus, HCN channels active during the AHP fail to completely deactivate as the membrane potential returns to the steady-state (Figures 7 and 8). As a result, HCN channels briefly introduce a small bias current that substantially increases the probability of initiating a subsequent action potential (Figure 8). To the best of our knowledge this is a unique function of HCN channels that depends critically upon both their activation by membrane hyperpolarization and their deactivation kinetics (Figures 7, 8, and S9). Such an interaction, between a bias current introduced by a slowly gating ionic current with a small single channel conductance such as I_K and rapidly varying currents composed of ion channels with larger single channel conductances, may be a general mechanism by which neurons produce changes in firing properties that pattern action potential output. Importantly, under naturalistic stimulus conditions, patterned spiking in the stochastic model can still provide significant modifications to the response properties of stellate neurons (Figure 9). Several neuronal subtypes have been reported to display perithreshold oscillations of membrane potential [58,59]. If intrinsic oscillations in other neurons also arise from stochastic channel gating, then patterned action potential firing driven by the interaction between multiple stochastic currents may also be a more general feature of neuronal spiking.

Relevance of Stochastic Channel Gating to Activity In Vivo

The entorhinal cortex is the last stage at which cortical information is processed prior to entering the hippocampal formation. Stellate cells in layer II constitute a major excitatory projection to the dentate gyrus and may correspond to the recently discovered ‘grid cells’, which encode an animal’s location in its environment through grid-like spatial firing fields [49,60,61]. While unreliable synaptic transmission is often considered as a noise source in neural circuits [6], less attention is usually given to the possible impact of stochastic channel fluctuations. Using stimulus parameters selected to obtain output firing properties similar to those recorded in vivo, we found that the presence of stochastically gating ion channels reliably increased the number of action potentials emitted with an ISI characteristic of intra-cluster intervals (Figure 9). This tendency depended on the stimulus statistics used, but is consistent with the peak in the in vivo ISI histograms around 100 ms and with our explanation for clustering as a transient increase in spike probability during the ~70–150 ms following an action potential. Since the trains of synaptic stimuli used for these simulations have random statistics, these data support the idea that the effects of stochastic ion channel gating
may in some conditions be superimposed on, rather than overwhelmed by, synaptic noise sources. Thus, stochastic ion channel gating may have to be accounted for in order to explain the firing of grid cells in behaving animals. However, further evaluation of this hypothesis will require much more information about the actual synaptic inputs received by grid cells. In addition, to better compare in vitro and in vivo data future studies will be required to establish whether in vivo data sets obtained from superficial layers of the MEC are indeed enriched for stellate neurons [49,60].

We also attempted to predict the responses of wild-type and HCN1 knockout neurons to naturalistic stimuli. These simulations suggested that in the absence of changes to the input stimulus, stellate neurons lacking HCN1 will have an approximately 65% reduction in average firing rate (Figure 9). This reduced firing rate is characterized by an increase in the fraction of spikes emitted in high frequency bursts, or a thinning of the response properties. There have been several suggestions that high frequency bursts convey unique information [62–64] about input stimuli and thus, this change could contribute to the enhancement of hippocampus-dependent learning in mice with deletion of HCN1 channels [28].

Conclusion

Whereas initiation of action potentials in deterministic model neurons is a binary process with a clearly definable threshold, in more realistic neuronal models containing stochastically gating ion channels spike initiation is probabilistic. Here we show that one general consequence of stochastic ion channel gating is that firing of an action potential can transiently modify the spike probability leading to the emergence of intrinsically generated patterns of spike output. In the case of the model we develop here, activation of HCN channels, during recovery from the action potential afterhyperpolarization, drives a brief increase in spike probability that leads to the emergence of clustered patterns of spike firing. As well as providing an account of both the resting and active integrative properties of stellate neurons in the medial entorhinal cortex, analysis of responses of this model to simulated in vivo synaptic inputs, suggests conditions in which stochastic ion channel gating might impact firing patterns of behaving animals. Thus, our results suggest a mechanism by which random changes in the conformation of small numbers of individual ion channel proteins could impact neural computations that underlie cognitive processes such as spatial navigation and memory.

Materials and Methods

Model Implementation

Modeling experiments were implemented in Matlab 7 (Natick, MA) using kinetic formalisms described in Text S1. The model has also been completely replicated in NEURON 5.9, but Matlab simulations were used for the data reported. The model cell was a sphere with a diameter of 50 μm and a specific capacitance of 1.67 μF/cm² (to account for the lack of a dendritic arbor). The model included implementations of a fast, transient sodium current (NaT), a persistent sodium current (NaP), a delayed rectifier-type postssium current (Kdr), a fast inactivating A-type potassium current (KaF) and a slowly inactivating potassium current (KaS), a “calcium-activated” potassium current (KCa), a linear potassium leak (Kl) and a fast or slow hyperpolarization-activated current (Hf or Hs). Hf, Hs and KCa are implemented as two-state channels, which is sufficient to capture their dominant kinetics, although additional states would be required to more fully capture detailed of their gating. NaP, KaF, and KaS, were modeled with a cyclical four state inactivation model. NaT and Kdr currents were modeled according to the original Hodgkin-Huxley formalism with 5 and 8 states, respectively. The total current density of each channel was closely matched to existing data.

In order to model stochastic channels, it was assumed that the states obeyed a first order Markov-type probabilistic description [2]. To track channel populations in each state a random number was generated for each channel in a given state (a “particle”) at each time step (Δt). Assuming that the time step is sufficiently small the probability of a transition is equal to rate × Δt, with a transition occurring in the event that a random number, evenly distributed between 0 and 1 is less than rate × Δt. For particles with multiple possible transitions (i.e., multistate channels that have multiple transitions into and out of a given state), a unique transition was chosen using non-overlapping distributions of transition probabilities. Briefly, a uniformly distributed collection of random numbers between zero and one, thresholded by the value P (transition) will give N, the number of transitions that occur. In the case where multiple transitions are possible, we observe that a given “particle” can only undergo a single transition. We know from probability theory that:

$$P(A \cup B) = P(A) + P(B) - P(A \cap B)$$

However, if there can only be a single transition then:

$$P(A \cap B) = 0$$

and thus,

$$P(A \cup B) = P(A) + P(B)$$

The probability that a given transition occurs is then the sum of the elementary probabilities. Dividing the probability space between 0 and 1 into bins of size P(A), P(B) and 1- P(A∪B), and placing random variables uniformly distributed between 0 and 1, gives the desired values for the number of transitions. This brute force method is similar to the simple Monte Carlo method described elsewhere [65] and to the method used elsewhere to model stochastic channels [9].

The time step used was 10 μs (corresponding to the approximate minimum dwell time of NaT) and numerical integration was accomplished using a 4th order Runge-Kutta method (most results were confirmed using the Backward Euler integration method). Simulations were run in Matlab and all analysis was completed using Igor Pro (Wavemetrics; Eugene, OR). A complete description of parameters used for the model currents and justification of parameters can be found in Text S1. Further, each channel was implemented as either stochastic or deterministic and it was ensured that in all cases the two solutions converged. For some simulations a partially stochastic model was used to speed simulation times and provide a good estimate of the fully stochastic model (data in Figures 4 and 9). This was justified by directly examining the contribution of each conductance (Figures S4 and S6).

Definitions

Throughout we have made reference to a number of descriptions of the biophysical properties of the neuron that are elaborated upon here for clarity. The passive membrane properties we characterize are the resting membrane conductance and resting membrane potential. Typically these values are obtained by analyzing the response of the membrane potential...
to small current steps. By convention we assume that the state of
the voltage-dependent currents is unaltered. The values are then
obtained by application of Ohm’s Law. However, during active
states, when the neuron or model is depolarized away from its
resting potential, the assumption that the underlying conductances
are unaltered by small changes in injected current are generally
less safe. At depolarized potentials we use a modified definition of
the membrane conductance and consider the “effective” mem-
brane conductance. Here we define the effective membrane
conductance as the slope of the relationship between the
membrane current and the membrane potential. This definition
thus explicitly takes in to account the change in membrane
conductance in response to a change in membrane voltage [2,5].

Analysis
All simulation data were analyzed in IGOR Pro (Wavemetrics)
using both built-in analysis functions and custom written routines.
Unless indicated otherwise mean values are ± standard error of the
mean (SEM). Statistical tests were accomplished using Excel
(Microsoft) and IGOR Pro (Wavemetrics).

Analysis of perithreshold fluctuations in membrane
potential. To analyze the spectral properties of perithreshold
fluctuations in membrane potential the built-in sonogram function
of IGRO Pro was used to estimate the short-time fourier transform
(STFT) of the membrane potential response to 20 s epochs of DC
current injection to the model. We further used the fast Fourier
transform (FFT) function to determine the spectral properties for
the entire epoch and selected 1 s sub-epochs of the response. For
the full 20 s analysis the FFT result was smoothed using a
Savitzky-Golay algorithm (35 point) for improved display.
Representative epochs were chosen from the central 10 s of data
based upon the appearance of coherent oscillatory behavior and
consistent with the changes in the power spectrum observed by
analyzing all such brief epochs. To compare the amplitude of
oscillations the mean of the integrated power spectra between 5–
10 Hz was calculated for all epochs.

Analysis of spike patterning. To quantify the tendency for
neurons to generate clustered patterns of spikes, we used previous
‘relaxed’ and ‘stringent’ definitions [18] for the data in Figure 4.
Subsequently, we used a single intermediate definition (400 ms
intercluster interval) to allow a single value to be reported where
helpful. Thus, a cluster of spikes was defined as two or more
consecutive spikes with interspike (intracluster) interval <250 ms,
preceded and followed by silent periods (intercluster intervals) of
duration >300 ms (relaxed), 400 ms (intermediate), or 500
(stringent) ms. We estimated the probability that a spike occurs
as part of a cluster (Pc) from the ratio of the number of spikes that
occur within clusters to the total number of spikes. Data were
binned according to the average firing rate (inverse of the mean
interspike interval) for all spikes during the entire 150 s simulation
or for each repetition of a 16 s trial (Figure 4D). Following the data
in Figure 4D, all subsequent analysis used the single, intermediate
definition of clustering.

Generation of P(s0|s0) distributions. We collected all
spike triggered membrane potential epochs for each simulation by
thresholding the first derivative of the membrane potential. The
spikes were aligned such that t = 0 at the threshold crossing, which
was operationally defined as 10% of the maximum of the first
derivative of the membrane potential. Using this ensemble of
spike-triggered membrane potential epochs we detected spikes
(using the same thresholded derivative) following the initial aligned
spike to create a spike-triggered raster plot. The rasters were then
binned (10 ms bins) and divided by the number of traces in the
ensemble to generate a spike-triggered spike probability

distribution as a function of time, t, following the time of the
aligned spike, t0, or “P(s0|s0)”. Estimate of passive membrane properties. Simulations
of responses to current steps (amplitude ± 5 pA; duration 5 s) were
run to estimate passive parameters of the stellate models. Input
resistance (Ri) was defined as the ratio of the steady state voltage in
response to positive (“+) or negative (“−”) current injection to the
resting potential. Monoexponential fits to the initial voltage
response were used to obtain the membrane time constant (τm).
The sag ratio is calculated as the ratio of the peak instantaneous
voltage difference divided by the steady-state voltage difference for
the negative current injection.

Experimental Data from In Vivo Recordings
Analysis of in vivo recordings of cortical neurons from the
superficial layers of the medial entorhinal cortex was based upon
data obtained from: http://commonweb.ntnu.no/cbm/moser/
gridcell.

Synaptic Stimulation
For Figure 9 we attempted to provide a general, readily
parameterized model of synaptic drive that might occur in vivo.
Because we used a single compartment model, appropriately
scaled current stimulation can be equivalent to conductance-based
stimuli [66]. Further, in order to provide a readily parameterized
stimulus to explore the space of possible responses we chose to use
colored white noise. Again, over the range of frequencies where
the impedance of the cell membrane is maximal, random barrages
of synaptic input show approximately white stimulus statistics [66].
For our stimulus we thus create a broadband, white noise stimulus
that was bandlimited to 50 Hz. The standard deviation and DC
offset of the current stimulus were scaled according to the
parameters in Figure 9A and 9B and applied directly to the model.

The ISI histogram of responses to the broadband stimulus was not
as broad as for the in vivo experimental data. Examining the
experimental data revealed that this was primarily due to a lack of
high-frequency (>50 Hz) bursting in the model. We made the
assumption that occasional changes in stimulus statistics could give
rise to this high frequency bursting. By examining the approximate
length of such periods we determined that ~200 ms long changes in
stimulus statistics were consistent with the experimental data.
We assumed a Poisson distribution for the duration of these epochs
of high frequency activity. We chose an interval between the high
frequency epochs that gave an approximately correct balance in
the ISI distribution (mean = 1 s; Poisson distributed). Finally, the
amplitude of the changes in the DC component and standard
deviation were taken from the survey of parameter space to match
the central peak of the bursting ISIs (see Figure 9).

Supporting Information
Figure S1 Stochastic gating can produce substantial channel
noise Found at: doi:10.1371/journal.pcbi.1000290.s001 (0.70 MB PDF)
Figure S2 Membrane impedance determines the increased
membrane potential fluctuations in the HCN1 knock-out model
Found at: doi:10.1371/journal.pcbi.1000290.s002 (3.38 MB PDF)
Figure S3 Ih is not required for perithreshold oscillations
Found at: doi:10.1371/journal.pcbi.1000290.s003 (2.78 MB PDF)
Figure S4 Necessity and sufficiency of stochastic conductances
Found at: doi:10.1371/journal.pcbi.1000290.s004 (4.69 MB PDF)
Figure S5 Spiking properties of the deterministic model
Figure S6  Partially stochastic model does not significantly differ from completely stochastic model

Figure S7  Voltage threshold for spike initiation is not correlated with ISI

Figure S8  Determining the critical point for spike initiation

Figure S9  A wide range of HCN kinetics are sufficient for AHP enhancement

Figure S10  A regularly spiking MEC stellate neuron

References


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Author Contributions

Conceived and designed the experiments: JTD MFN. Performed the experiments: JTD MFN. Analyzed the data: JTD MFN. Contributed reagents/materials/analysis tools: JTD MFN. Wrote the paper: JTD MFN.