An integrate-and-fire approach to Ca$^{2+}$ signaling. Part I: Renewal model

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ABSTRACT In computational neuroscience integrate-and-fire models capture the spike generation by a subthreshold dynamics supplemented by a simple fire-and-reset rule; they allow for a numerically efficient and analytically tractable description of stochastic single cell as well as network dynamics. Stochastic spiking is also a prominent feature of Ca$^{2+}$ signaling which suggests to adopt the integrate-and-fire approach for this fundamental biophysical process. The model introduced here consists of two components describing 1) activity of clusters of inositol-trisphosphate receptor channels and 2) dynamics of the global Ca$^{2+}$ concentrations in the cytosol. The cluster dynamics is given in terms of a cyclic Markov chain, capturing the puff, i.e., the punctuated release of Ca$^{2+}$ from intracellular stores. The cytosolic Ca$^{2+}$ concentration is described by an integrate-and-fire dynamics driven by the puff current. For the cyclic Markov chain we derive expressions for the statistics of the interpuff interval, the single-puff strength and the puff current assuming constant cytosolic Ca$^{2+}$. The latter condition is often well approximated because cytosolic Ca$^{2+}$ varies much slower than the cluster activity does. Furthermore, because the detailed two-component model is numerically expensive to simulate and difficult to treat analytically, we develop an analytical framework to approximate the driving puff current of the stochastic cytosolic Ca$^{2+}$ dynamics by a temporally uncorrelated Gaussian noise. This approximation reduces our two-component system to an integrate-and-fire model with a nonlinear drift function and a multiplicative Gaussian white noise, a model that is known to generate a renewal spike train, i.e., a point process with statistically independent interspike intervals. The model allows for fast numerical simulations, permits to derive analytical expressions for the rate of Ca$^{2+}$ spiking and the coefficient of variation of the interspike interval, and to approximate the interspike interval density and the spike train power spectrum. Comparison of these statistics to experimental data is discussed.

INTRODUCTION

Calcium (Ca$^{2+}$) is a ubiquitous intracellular messenger that regulates many cellular processes, ranging from muscle contraction and relaxation over neurotransmitter secretion in synapses to fertilization and apoptosis (1–5). To utilize Ca$^{2+}$ signaling effectively, cells maintain an extraordinary low resting cytosolic Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_i \ll 100 \text{ nM}$) much smaller than the extracellular Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_\text{ex} \approx 1 \text{ nM}$) (6). The associated large gradient is maintained by a number of pumps that transport Ca$^{2+}$ from the cytosol into the extracellular medium but also into the endoplasmic reticulum (ER) serving as an intracellular Ca$^{2+}$ store with a lumenal concentration three to four orders of magnitude larger than the cytosolic resting concentration. Release of Ca$^{2+}$ from the ER through the designated inositol triphosphate receptor (IP$_3$R) channel and controlled by the second messenger inositol trisphosphate (IP$_3$) causes

SIGNIFICANCE The concentration of intracellular Ca$^{2+}$ controls many cellular processes and exhibits prominent pulses (spikes). The timing of these spikes is rather irregular due to an interplay of the intracellular Ca$^{2+}$ dynamics with the random opening and closing of Ca$^{2+}$ channel clusters in the membrane of the endoplasmic reticulum. Here, we propose a mathematical model for the random (stochastic) spike generation that incorporates these two components. Since cluster activity is often very fast compared with the change in Ca$^{2+}$ concentration, we can further reduce our two-component model resulting in a simplified stochastic model that can be rapidly simulated. Moreover, the simpler model allows for analytical approximations of the statistics of interest, which can be compared with those of experimental data.
short pulse-like deflections of the cytosolic Ca\(^{2+}\) concentration called spikes.

Ca\(^{2+}\) spiking is a stochastic process (7–17). The sequence of spike times is strongly affected by randomness due to the finite-size noise of the involved molecules as well as cooperativity of the ion channels (see below). Theoretical understanding of Ca\(^{2+}\) signaling requires stochastic models that take these fluctuations into account. Such models exist (9,18–22) but so far do not focus on the spike statistics as a point process. In computational neuroscience an important model class for the description of a stochastic point process, associated with the spiking in the membrane voltage of neurons, is the integrate-and-fire (IF) neuron (23–26). Here, we attempt to adopt this successful framework to the problem of Ca\(^{2+}\) spiking; for a recent discussion of the similarities and differences between calcium and neural spiking (see, e.g., (27)).

The defining idea of an IF approach is to model the dynamics of the spiking variable (in computational neuroscience the membrane voltage) only up to its spike-triggering threshold, and to replace the stereotypical spike by a fire-and-reset rule. The most important output of such a model is the sequence of spike times \(t_i\) or, equivalently, the spike train:

\[
z(t) = \sum_i \delta(t - t_i),
\]

a sum of Dirac delta functions \(\delta(\cdot)\) at the spike times. IF models are successful in reproducing and predicting the spike times of detailed neuron models (26) and real neurons (28,29) alike and are analytically tractable in many situations (see, e.g., (30–35)).

Crucial for the applicability of IF models is a reliable spike generation once the threshold is crossed. This in turn is implemented by a positive feedback mechanism, based on voltage-gated ion channels that are selective for Na\(^+\) ions. Superthreshold depolarization of the voltage opens Na\(^+\) channels triggering a Na\(^+\)-flux into the cell, that leads to a further depolarization of the voltage and activates even more Na\(^+\) channels and so on. Ca\(^{2+}\)-induced Ca\(^{2+}\) release, a process in which Ca\(^{2+}\) itself promotes the release of Ca\(^{2+}\) from the ER, constitutes such a positive feedback mechanism in the context of Ca\(^{2+}\) spiking.

The model we propose here consists of two components. The first describes the dynamics of the intracellular Ca\(^{2+}\) concentration by means of an IF model. The second captures the release of Ca\(^{2+}\) from the ER by the stochastic opening of clusters of IP\(_3\)R's (modeling channel activity by Markovian models has a long tradition, for an overview see (21)). These local releases are called puffs and form the elementary events of global spikes. We describe them by a cyclic Markov chain modeling the number of open channels in a given cluster. The Ca\(^{2+}\) puff current through these channels results from the concentration gradient between ER and cytosol. We calculate statistics of the puff strength and interpuff interval (IPI) as well as the noise intensity of the puff current from the Markov chain.

The model for the global intracellular Ca\(^{2+}\) concentration is hard to treat analytically because the driving puff current depends on the cytosolic Ca\(^{2+}\) concentration itself and possesses complicated statistics. However, because the opening and closing of IP\(_3\)R clusters is often fast, we exploit the timescale separation between the fast IP\(_3\)-dependent puff current and the remaining slow IP\(_3\)-independent Ca\(^{2+}\) currents. This allows for a diffusion approximation of the puff current and reduces the two-component model to a one-component IF model with nonlinear drift and Gaussian white noise. This reduced model generates a renewal point process, i.e., all interspike intervals (ISIs) are mutually statistically independent; this is also true to a very good approximation for the original two-component model. The reduced one-component model is analytically tractable and allows to calculate statistics such as the stationary probability density of the subthreshold Ca\(^{2+}\) concentration, the firing rate, and the coefficient of variation (CV) of the ISI. We find, that for our model the ISI density is well approximated by an inverse Gaussian distribution which for the generated renewal point process provides a complete description of the spike statistics and allows for an instance to calculate the spike-train power spectrum. We show that the model can reproduce quantitatively statistics of stimulated HEK cells that have been shown to generate renewal spike trains (36).

The paper is organized as follows. We first introduce the two-component model for Ca\(^{2+}\) spiking starting with the IF model that governs the dynamics of the subthreshold cytosolic Ca\(^{2+}\) concentration. In the following section we introduce the cyclic Markov chain that models the cooperative opening and closing of IP\(_3\)R clusters and gives rise to a stochastic Ca\(^{2+}\) current that feeds into the cytosolic Ca\(^{2+}\) concentration. Before discussing the full two-component model we study the Markov chain for a fixed cytosolic Ca\(^{2+}\) concentration and provide exact analytical results for statistics of the puff strength and IPI as well as the mean and noise intensity of the puff current. In the subsequent section we approximate the stochastic puff current by a deterministic mean and Gaussian white noise, based on a timescale separation between the Ca\(^{2+}\) currents. This gives rise to a one-component nonlinear Langevin equation with a spike-and-reset rule, that can be treated analytically by means of the associated Fokker-Planck equation (FPE). Finally, we compare statistics of the full two-component model to those of the one-component Langevin approximation and also to stimulated HEK cells. For an overview of the introduced variables and parameters see Table 1.

### Two-component model for cytosolic Ca\(^{2+}\) and stochastic IP\(_3\)R cluster dynamics

**Cytosolic Ca\(^{2+}\): IF model**

We introduce an IF modeling approach to the repetitive spiking of [Ca\(^{2+}\)]. In such a framework the dynamics of
Over this range the change of the (nondimensional) cytosolic [Ca]i is modeled only over a small part of its physiological range, i.e., up to a certain threshold that is much smaller than the peak concentration during a spike. We assume that the positive feedback mechanism known as Ca2+-induced Ca2+ release of Ca2+ from the ER by clustered IP3Rs. We describe j_{puff} as:

$$j_{puff}(c_i) = p \Delta c_{er} \sum_{k=1}^{K} x_k(t),$$

where $p$ is a permeability-like parameter, $\Delta c_{er}$ is the concentration gradient between ER and cytosol, and $x_k(t)$ refers to the number of open channels in the $k$th of a total of $K$ clusters. The kinetics of $x_k(t)$ are detailed in the following section. Since the Ca2+-concentration in the ER is much larger than in the cytosol (5,21), we assume $\Delta c_{er}$ to be independent of $c_i$ and constant in time. New aspects arising from a dynamic luminal concentration will be explored in a subsequent publication.

The IF model is completed by a fire-and-reset rule: at each time $t_i = t$ when $c_i(t)$ reaches the threshold $c_T$, a spike is assumed to occur and $c_i$ is reset to $c_R$. If we want to include a finite width of the Ca2+ spike we could (similarly to what is done in neuroscience) clamp the Ca2+ concentration to $c_R$ for an absolute refractory period $\tau_{abs}$. For simplicity we chose in this paper $\tau_{abs} = 0$ and $c_R = c_0$.

The so far described IF component of our model is illustrated in Fig. 1 B. The experimental data shown in Fig. 1 A can be mimicked by the model if the parameters are chosen accordingly such that the model shows similar values for the stationary mean and CV of the ISI $T_i$ (in the following for stationary statistics of the ISI we will omit the index $i$); this is further discussed below.

**IP3R clusters: Cyclic Markov model**

The number of open channels in each cluster, $x(t)$ (omitting the cluster index $k$), is modeled by a cyclic Markov chain that is shown in Fig. 2 (top left). The model is based on experimentally accessible statistics of the puff strength and IP3. There exists of course a large variety of detailed single IP3R models upon which a cluster model could be build (39), for instance, the classic De Young-Keizer model (40) or the more recent data-driven models by Gin et al. (41) and Siekmann et al. (42), to name just a few. However, because channels within a cluster are highly cooperative, indicating strong coupling, cluster models derived from single-channel-state schemes are very complex (43,44). Since these complex dynamics result in rather simple waiting time distributions for closed and open cluster states (43,45–47), we follow here a different approach.

For our model each cluster is described by a continuous-time Markov chain $\omega(t)$ with state space $\Omega = [N, …, 1, 0_M, …, 0]$ that consists of two subsets $\Omega_N = [N, …, 1]$ and $\Omega_M = [0_M, …, 0]$ where the cluster is considered open and closed, respectively (see Fig. 2). The states are labeled according to the number of open channels they represent and we refer to the probability

<table>
<thead>
<tr>
<th>TABLE 1 Variables and parameters</th>
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<tbody>
<tr>
<td>Symbol</td>
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<tr>
<td>$c_i(t)$</td>
</tr>
<tr>
<td>$c_0$, $c_R$, $c_T$</td>
</tr>
<tr>
<td>$\tau$</td>
</tr>
<tr>
<td>$j_{puff}(c_i)$</td>
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<tr>
<td>$p$</td>
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<tr>
<td>$\Delta c_{er}$</td>
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<tr>
<td>$x_k(t)$</td>
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<tr>
<td>$K$</td>
</tr>
<tr>
<td>$N$</td>
</tr>
<tr>
<td>$M$</td>
</tr>
<tr>
<td>$\lambda_{abs}$, $\lambda_{puff}$, $\lambda_{reset}$</td>
</tr>
<tr>
<td>$a$, $b$</td>
</tr>
<tr>
<td>$s$</td>
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that a state $\omega$ is occupied by $p(\omega, t)$ or equivalently by the $\omega$th component of a probability vector $p(t)$. The master equation is given by:

$$\frac{d}{dt}p(t) = Wp(t), \quad (4)$$

with the transition rate matrix $W$:

$$
\begin{pmatrix}
-\lambda_{\text{cls}} & \lambda_{\text{ops}}/N \\
\lambda_{\text{cls}} & -\lambda_{\text{cls}} \\
\vdots & \vdots \\
\lambda_{\text{ref}} & -\lambda_{\text{ref}} \\
\lambda_{\text{ops}}/N & \lambda_{\text{ops}}/N \\
\end{pmatrix}
\quad (5)
$$

where each blank entry corresponds to a zero, and the probability vector $p(t)$:

$$
(p(N, t) \quad p(1, t) \quad p(0_M, t) \quad p(0_1, t))^T \quad (6)
$$

Furthermore, we assign to every state $\omega$ a natural number as follows:

$$x(\omega) = \begin{cases} 
 n, & \text{for } \omega \in \Omega_N, \\
 0, & \text{for } \omega \in \Omega_M. 
\end{cases}$$

Here, $n$ is the number of channels that are currently open and corresponds to the label of the state, for instance, $x(\omega = 3) = 3$. The corresponding random process $x(t)$ hence represents the number of open channels at time $t$ and enters the Ca$^{2+}$ dynamics according to Eq. 3. The transition rate matrix $W$ consists of four submatrices that arise due to the classification of the state space into sets of open and closed states. The shapes of the submatrices and their biophysical rationales are described in the following, starting with the top right submatrix and continuing counterclockwise. The first three submatrices determine statistics of the puff (see Fig. 2 A), in particular the puff strength $A$, defined as the integrated Ca$^{2+}$ current during a single puff, while the fourth submatrix determines statistics of the time between two successive puffs, the IPI $I$ (see Fig. 2 B).

The top-right $N \times M$ submatrix captures transitions from the closed state $\omega = 0_1$ to the open states and thus the opening of the cluster. Biophysically the firing of a puff is a highly cooperative event triggered by the opening of a single channel which greatly increases the local Ca$^{2+}$ concentration. This in turn increases the open probability (48) of the remaining channels in the same cluster and causes a certain, uniformly distributed number of them to respond, i.e., to open as well (49,50). In our model we consider this response to be instantaneous so that the passage from $\omega = 0_1$ (closed) to any number of open channels $\omega = n_0$ (open) is described by single transition. The total escape rate from $0_1$, $\lambda_{\text{ops}}$, depends linearly on the cluster size $N$, which has been confirmed experimentally (51) and reflects the idea that the opening of any channel can serve as a puff trigger (see Eq. 7). Furthermore, in line with experimental findings (50), we assume that the
corresponding efflux of probability splits equally between all open states, so that the transition rate to a particular open state is $\lambda_{\text{opn}}/N$. Indeed, as seen in Fig. 2 A2, the probability for the initial number of open channels in a puff, referred to as the number of responding channels $n_0$ in the following, is uniformly distributed over the $N$ states.

Once a puff is initiated, the channels close successively according to the top-left $N/M$ (and bottom-left $M/N$)
submatrix. Intuitively, one could assume that the channels in a cluster close independently and that the closing rate of the cluster therefore depends linearly on the number of channels \( n \) that are currently open. However, Wiltgen et al. (52) have shown that the mean dwell time, the inverse of the closing rate, is largely independent of \( n \) indicating that channels do not close independently, but the details of this process are poorly understood. Accordingly, in our model we describe the transition from \( \omega = n \) to \( \omega = n - 1 \) by a fixed closing rate \( \lambda_{\text{cls}} \) that is independent of \( n \); an example of the corresponding staircase-like shape of the puff is shown Fig. 2A1. The bottom-left \( M \times N \) submatrix, containing only a single entry, describes the closing of the last channel, i.e., transitions from \( \omega = 1 \) to \( \omega = 0 \).

The bottom-right \( M \times M \) submatrix determines statistics of the IPI. Experimentally, it is known that IPIs are highly stochastic but display a (small) refractory period (45,51). The latter can be understood by a negative feedback emerging during the opening of the channel: the local \( \text{Ca}^{2+} \) concentration in channel proximity attains very large values within microseconds upon channel opening (53) and, after closing of the channel, reduces the probability of immediate reopening for a relative refractory period (48,54). While this feedback is a subject of ongoing discussion, even studies which consider such feedback unlikely come to the conclusion that the IP\(_3\)R has more than one closed state (55). IPIs can thus not be modeled by a single transition that would result in an exponential IPI distribution. We introduce a number of refractory states which have to be traversed after a puff was fired before a new puff can be initiated. In detail, the first \( M - 1 \) closed states have to be traversed with transition rates \( \lambda_{\text{ref}} \) before the cluster can open again with the respective opening rate \( \lambda_{\text{opn}} \). At this point, upon reaching the state \( \omega = 0 \), the cycle starts anew with the opening of the cluster captured by the top-right submatrix.

Finally, to complete the model, we state our assumption about the \( \text{Ca}^{2+} \) and IP\(_3\) dependence of the rates. Unfortunately, an extraction of these rates from experimental data is, to the best of our knowledge, not feasible—a quantitative study that relates puff activity to [\( \text{Ca}^{2+} \)] is missing. For a lack of direct measurements, we choose the open rate’s \( \lambda_{\text{opn}} \) \( \text{Ca}^{2+} \) dependence in analogy to the biphasic \( \text{Ca}^{2+} \) dependence of an individual channel’s open probability, usually described by a combination of Hill equations (48,56,57). Since we utilize an IF modeling approach, we are only interested in cluster activity during the rising phase of the \( \text{Ca}^{2+} \) spike and do not include a term that captures the decrease of the rates at high concentrations. We choose:

\[
\lambda_{\text{opn}}(c_i) = N\lambda_{\text{opn}} \frac{c_i^\alpha}{1 + c_i^\beta} \frac{s^\gamma}{1 + s^\delta},
\]

where \( c_i = [\text{Ca}^{2+}]/K_{\text{act}} \) and \( s = [\text{IP}_3]/K_{\text{stim}} \) refer to the cytosolic \( \text{Ca}^{2+} \) and \( \text{IP}_3 \) concentration relative to their dissociation constants \( K_{\text{act}} \) and \( K_{\text{stim}} \) at which the rates attain half of their maximal value.

The remaining rates of our model \( \lambda_{\text{ref}}, \lambda_{\text{cls}} \) do not depend on \( \text{Ca}^{2+} \) nor on IP\(_3\). The rationale for this choice is a follows: the time for a single channel to be open was shown to be largely independent of \( \text{Ca}^{2+} \) (56), hence it is plausible that the rate \( \lambda_{\text{cls}} \) is unaffected. Furthermore, as discussed above, the local \( \text{Ca}^{2+} \) concentration after a puff, i.e., during passage of the refractory states, bears little relation to the global \( \text{Ca}^{2+} \) concentration and thus the rates in the refractory states \( \lambda_{\text{ref}} \) may be assumed to be independent of \( \text{Ca}^{2+} \). In conclusion we simply assume:

\[
\lambda_{\text{ref}} = \text{constant},
\lambda_{\text{cls}} = \text{constant}.
\]

The resulting open probability of a single cluster is given by:

\[
p_{\text{opn}}(c_i) = \frac{\tau_{\text{opn}}}{\tau_{\text{tot}}(c_i)} = \frac{\tau_{\text{opn}}}{\tau_{\text{opn}} + \tau_{\text{cls}}(c_i)} = \frac{1}{1 + \frac{N - 1}{2\lambda_{\text{cls}}}} \frac{1}{\lambda_{\text{ref}} + \frac{1}{\tau_{\text{opn}} + \tau_{\text{cls}}(c_i)}},
\]

where \( \tau_{\text{opn}} = \frac{N \lambda_{\text{opn}}}{1 - \lambda_{\text{act}}} \) and \( \tau_{\text{cls}} = (M - 1)/\lambda_{\text{cls}} + 1/\lambda_{\text{ref}} \) are the mean residence times in the open and closed states, respectively, and \( \tau_{\text{tot}} = \tau_{\text{opn}} + \tau_{\text{cls}} \) is the total mean period of the cyclic Markov chain. We note that, \( \tau_{\text{cls}} \) corresponds to the mean IPI and \( \tau_{\text{opn}} \) is the mean puff duration. The resulting open probability and the mean times are shown in Fig. 3 as functions of \( c_i \). We indicate by the gray area the \( c_i \) range that is inaccessible in our IF framework, which does not capture the spike generation itself but only the signal- and noise-dependent subthreshold dynamics. To make a connection to works that cover the full range, we also show a modified open probability with the full biphasic \( c_i \) dependence obtained by multiplying the opening rate in Eq. 7 by a factor \( k^a/(k^a + c_i^a) \) with \( k = K_{\text{inh}}/K_{\text{act}} \). It becomes apparent, that there is no effect of this inhibitory term for the subthreshold \( c_i \) relevant in our model.

Equation 2 for the IF component and Eq. 4 for the \( K \) clusters of IP\(_3\)R channels constitute the complete model that can be simulated by the typical integration schemes for differential and master equations.

**RESULTS**

**Statistics of a single IP\(_3\)R cluster for fixed Ca\(^{2+}\): Analytical results**

Here, we calculate statistics of the puff strength \( A \) and IPI \( I \). Because the \( \text{Ca}^{2+} \) concentration usually changes very slowly compared with the cluster activity, we assume that
c_i is fixed. In this case and I are fully independent and can be calculated separately.

We start with the puff strength A, i.e., the integrated Ca^{2+} current during a single puff or, in biophysical terms, a quantity proportional to the amount of Ca^{2+} released per puff. As stated previously, the closing rate is independent of the number of open channels. As we will see, a consequence of this is that the mean puff strength \( \langle A \rangle \) is a quadratic function of the number of IP_3Rs in a cluster N as demonstrated in Fig. 2 A. In contrast to that, the CV of the puff strength, CV_A, is largely independent of N, see Fig. 2 A. Both findings can be understood within our analytical framework presented in the following.

To calculate the mean \( \langle A \rangle \), variance \( \text{Var}(A) \) and \( CV_A^2 = \text{Var}(A)/\langle A \rangle^2 \), it is helpful to first determine the conditional means \( \langle A|n_0 \rangle \) and variances \( \text{Var}(A|n_0) \), given a certain number of responding channels \( n_0 \), and subsequently to use the law of total mean and variance (58) to relate both to each other:

\[
\langle A \rangle = \langle \langle A|n_0 \rangle \rangle_{n_0},
\]

\[
\text{Var}(A) = \langle \text{Var}(A|n_0) \rangle_{n_0} + \text{Var}(\langle A|n_0 \rangle)_{n_0}.
\]

Here, we have explicitly emphasized over which quantity the average was formed but, in the following, will omit the indices for the ease of notation. We can consider the conditional puff strength \( \langle A|n_0 \rangle \) to be the sum of \( n_0 \) random numbers, namely the area under each “step” of the puff denoted by \( (a|n) \):

\[
\langle A|n_0 \rangle = \sum_{a=1}^{n_0} (a|n).
\]

For instance, for the first puff shown in Fig. 2 A, the number of responding channels is \( n_0 = 4 \) and the area under the puff \( A_1 \), can be considered to be the sum of four independent random numbers. For the \( n \)th step the area \( (a|n) = n \tau_n \) is the product of the (stochastic) dwell time \( \tau_n \) and the (deterministic) number of open channels \( n \). Because \( \tau_n \) is exponentially distributed, so is \( (a|n) \) with a rescaled rate \( \lambda_n = \lambda_{cls}/n \).

Since we deal with independent random variables their means and variances sum up and we find:

\[
\langle A|n_0 \rangle = \sum_{n=1}^{n_0} \langle a|n \rangle = \sum_{n=1}^{n_0} \frac{n}{\lambda_{cls}},
\]

\[
= \frac{1}{2}\lambda_{cls}(n_0 + 1),
\]

\[
\text{Var}(A|n_0) = \sum_{n=1}^{n_0} \text{Var}(a|n) = \sum_{n=1}^{n_0} \frac{n^2}{\lambda_{cls}^2},
\]

\[
= \frac{1}{6}\lambda_{cls}(n_0 + 1)(2n_0 + 1).
\]

The unconditional mean is readily obtained using Eqs. 10a and 12a as well as the fact that \( n_0 \) is uniformly distributed over \( N \):

\[
\langle A \rangle = \langle \langle A|n_0 \rangle \rangle_{n_0} = \frac{1}{N} \sum_{n_0=1}^{N} \frac{1}{2}\lambda_{cls}(n_0 + 1)
\]

\[
= \frac{1}{6}\lambda_{cls}(N + 1)(N + 2).
\]

It can be seen that the average puff strength is indeed a quadratic function of the number of channels \( N \) as pointed out above. The total variance can be calculated according to Eq. 10b using the conditional mean and variance in Eqs. 12a and 12b:

\[
\langle \text{Var}(A|n_0) \rangle = \frac{1}{12}\lambda_{cls}^2(N + 1)^2(N + 2),
\]

\[
\text{Var}(A|n_0) = \langle \text{Var}(A|n_0) \rangle_{n_0} + \text{Var}(\langle A|n_0 \rangle)_{n_0}.
\]
\[
\text{Var}(\langle A | n_0 \rangle) = \frac{1}{60 \lambda_{\text{cls}}} (N + 1) (N + 2) (3N^2 + 6N + 1) \\
- \frac{1}{56 \lambda_{\text{cls}}} (N + 1)^2 (N + 2)^2.
\]

(14b)

and adding the two together. This yields an expression that is cumbersome at first glance. However, it turns out that the CV:

\[
CV_A^2 = \frac{\text{Var}(\langle A | n_0 \rangle) + \text{Var}(\langle A | n_0 \rangle)}{\langle A \rangle^2} = \frac{4N^2 + 18N + 8}{5N^2 + 15N + 10}.
\]

(15)

is a monotonically decreasing function for \(N \in \mathbb{N}_{>0}\), bound between 1 and 0.8 as shown in Fig. 2 A\(_i\). The upper bound corresponds to a single channel (\(N = 1\)) where the puff is made of a single step with puff strength drawn from an exponential distribution, accordingly \(CV_A^2 = 1\). In this case only the first term on the r.h.s. of Eq. 10b contributes to the total variance, because there is no variability in the number of responding channels. The lower bound is obtained for infinite cluster sizes \(N \to \infty\): In this case the second term on the r.h.s. of Eq. 10b determines the total variance and we obtain

\[
\lim_{N \to \infty} CV_A^2 = \lim_{N \to \infty} \frac{\text{Var}(\langle A | n_0 \rangle)}{\langle A \rangle^2} = \frac{4}{5}.
\]

(16)

Hence, even for large clusters consisting of many IP\(_3\)R channels the variability of the number of responding channels ensures the strong variability of the puff strength. Fig. 2 A\(_i\) shows the contributions of \(\text{Var}(\langle A | n_0 \rangle)/\langle A \rangle^2\) (dotted line) and \(\text{Var}(\langle A | n_0 \rangle)/\langle A \rangle^2\) (dashed line) to the total (squared) CV as a function of the cluster size.

We now turn to the IPI \(I\), that is the time between two subsequent puffs (excluding the duration of the puff itself), see Fig. 2 B\(_j\). In contrast to the puff strength, the IPI statistics depends heavily on the chosen Ca\(^{2+}\) concentrations. Here we numerically illustrate our results for the resting and threshold Ca\(^{2+}\) concentrations.

The mean and variance of the IPI are readily obtained if the IPI is considered to be the sum of the exponentially distributed dwell times:

\[
I = \sum_{m=1}^{M} \tau_{0m}
\]

(17)

where \(\tau_{0m}\) are independent random times spend in the states \(\omega = 0_m\). It follows that:

\[
\langle I \rangle = \sum_{m=1}^{M} \langle \tau_{0m} \rangle = \frac{M - 1}{\lambda_{\text{ref}}} + \frac{1}{\lambda_{\text{opn}}},
\]

(18a)

\[
\text{Var}(I) = \sum_{m=1}^{M} \text{Var}(\tau_{0m}) = \frac{M - 1}{\lambda_{\text{ref}}} + \frac{1}{\lambda_{\text{opn}}^2},
\]

(18b)

We first discuss the obvious dependence on the number of closed states \(M\). Trivially, the mean IPI increases with \(M\) because the system has to traverse more states. In contrast, the variability decreases monotonically with the number of closed states, starting with a \(CV_I = 1\) for \(M = 1\) (corresponding to a Poisson process) and vanishes for large as \(CV_I \propto 1/\sqrt{M}\).

Another interesting question is how the statistics of the IPI depends on the cluster size \(N\) which affects the moments only through the opening rate \(\lambda_{\text{opn}} \propto N\), see Eq. 7. The inverse mean \(\langle I \rangle^{-1}\) is shown in Fig. 2 B\(_l\) and displays a close to linear shape when the Ca\(^{2+}\) concentration is at rest \(c_1 = c_0\) (solid line in Fig. 2 B\(_l\)). This is reasonable given that in the considered parameter regime in Eq. 18a the first term (independent of \(N\)) is much smaller than the second term (proportional to \(1/N\)), hence the inverse mean IPI is approximately proportional to \(N\). For higher Ca\(^{2+}\) concentrations, for instance, at the threshold \(c_1 = c_T\), the rate \(\lambda_{\text{opn}}\) is larger and the dependence of \(\langle I \rangle\) on \(N\) is more nonlinear (dashed line in Fig. 2 B\(_l\)).

The irregularity of the IPI is quantified by the \(CV_I\) and also depends on the cluster size through \(\lambda_{\text{opn}}\). Specifically, for small clusters the IPI is mainly determined by the dwell time in the state \(\omega = 0_1\) and, consequently, almost exponentially distributed with a \(CV_I\) close to one. For larger clusters, however, the state \(\omega = 0_1\) is left quickly, and the IPI is primarily determined by the concatenation of the refractory states which yields more regular intervals. However, at the resting Ca\(^{2+}\) concentration, there is little variation of the \(CV_I\) over a moderate range of cluster sizes \(N\) because the mean dwell time \(\tau_{01} = 1/\lambda_{\text{opn}}\) still dominates the IPI for all \(N\) (solid line in Fig. 2 B\(_l\)). A tendency toward lower values of the \(CV_I\) can indeed be observed for higher Ca\(^{2+}\) concentrations close to the threshold of Ca\(^{2+}\) spiking (dashed line in Fig. 2 B\(_l\)). For much larger opening rates \(\lambda_{\text{opn}}\), encountered at large cluster sizes, the \(CV_I\) saturates at \(CV_I = 1/\sqrt{M - 1}\) for \(M > 1\).

The full IPI probability density function \(p_{\text{IPI}}(t)\) is obtained as a convolution of \(M - 1\) exponential densities with a rate \(\lambda_{\text{ref}}\) and one exponential density with rate \(\lambda_{\text{opn}}\); this is so because the IPI is the sum of the statistically independent dwell times in the closed states. The result of the convolution integral can be expressed by the lower incomplete gamma function \(\gamma(a, t) = \int_0^t dt' t'^{a-1} e^{-t'}\).
\[ p_{\text{PI}}(t) = \lambda_{\text{opn}} p_0 (t) \]
\[ = \lambda_{\text{opn}} \left( \frac{\lambda_{\text{ref}}}{\lambda_{\text{ref}} - \lambda_{\text{opn}}} \right) (M - 1) \gamma (M - 1, \lambda_{\text{ref}} - \lambda_{\text{opn}}) t e^{-\lambda_{\text{opn}} t}, \]
\[ \text{Eq. 19} \]

a simple result confirmed for two different Ca\textsuperscript{2+} concentrations in Fig. 2, B2 and B3.

Most importantly, for the Langevin approximation derived below, we can calculate the mean \( \mu_x \) and noise intensity \( D_x \) of a single cluster \( x(t) \) by means of algebraic equations. One ingredient needed in following is the stationary probability vector of the states, determined by the stationary master equation and the normalization condition:
\[ 0 = W \cdot p_0, \text{ and } \sum_{i \in \Omega} p_0 (i) = 1, \]
\[ \text{Eq. 20} \]

where \( 0 \) is the zero vector. The solution of Eq. 20 reads for our specific model:
\[ p_0 (i) = \begin{cases} \frac{N + 1 - i}{N} \frac{1}{\tau_{\text{tot}} \lambda_{\text{cls}}}, & \text{if } i \in [N, \ldots, 1] \\ \frac{1}{\tau_{\text{tot}} \lambda_{\text{ref}}}, & \text{if } i \in [0_M, \ldots, 0_2] \\ \frac{1}{\tau_{\text{tot}} \lambda_{\text{opn}}}, & \text{if } i = 0_1 \end{cases} \]
\[ \text{Eq. 21} \]

This allows to calculate the mean of the puff current:
\[ \mu_x = \sum_{i \in \Omega} x(i) \cdot p_0 (i) \]
\[ = \frac{1}{6 \lambda_{\text{cls}}} \frac{(N + 1)(N + 2)}{\lambda_{\text{opn}} + \frac{M - 1}{\lambda_{\text{ref}}} + \frac{N + 1}{2 \lambda_{\text{cls}}}} = \langle A \rangle \frac{1}{\tau_{\text{tot}}}. \]
\[ \text{Eq. 22} \]

While the determination of the stationary probabilities and the mean follows standard procedures (59,60), the calculation of the noise intensity for the process \( x(t) \) is more advanced.

First of all, the noise intensity is defined by
\[ D_x = \int_0^\infty d\tau \ C_{xx}(\tau). \]
\[ \text{Eq. 23} \]

From this equation it is not so obvious to see that the noise intensity is determined by an algebraic equation. After all the correlation function, \( C_{xx}(\tau) = \langle x(t)x(t + \tau) \rangle - \langle x \rangle^2 \), requires the time-dependent solution \( p(t) \) of the master equation. However, we can use the fact that we only need the integral of the correlation function to avoid solving the time-dependent problem; a similar trick has been applied to calculate the correlation time (61) and the diffusion coefficient (62,63) of systems described by a FPE. To that end we express the correlation function in terms of transition and stationary probabilities:
\[ D_x = \int_0^\infty d\tau \ C_{xx}(\tau) \]
\[ = \int_0^\infty d\tau \ (x(t + \tau)x(t) - \langle x \rangle^2) \]
\[ = \int_0^\infty d\tau \ \sum_{i,j} [x(i)x(j) p(i, t + \tau|j, t) p_0 (j)] \]
\[ - \int_0^\infty d\tau \ \sum_{i,j} [x(i)x(j) p_0 (i) p_0 (j)] \]
\[ = \sum_{i,j} x(i)x(j) [p(i, t + \tau|j, t) - p_0 (i)] p_0 (j) \]
\[ = \sum_{i,j} x(i)x(j) f(i, j) p_0 (j). \]
\[ \text{Eq. 24} \]

Note that the sums run again over all possible states of a cluster. Formally, we still have to find the time-dependent transition probabilities \( p(i, f|j, t) \), however, it turns out that the auxiliary function \( f(i, j) \), that contains integrals of probability functions, is much simpler to calculate. In particular, the functions \( f(i, j) \) can be determined as the solution of an algebraic instead of a differential equation which simplifies the evaluation considerably. To make this clear consider the \( \ell \)-th row of the master equation Eq. 4 with the additional condition that the state at time \( t \) is known, i.e.,
\[ p(l, t) = \delta_{ij}; \]
\[ \int_0^\infty d\tau \ p(l, t + \tau|j, t) = \int_0^\infty d\tau \sum_i w_{lj} p(t + \tau|j, t) - p_0 (i) \]
\[ p_0 (l) - \delta_{ij} = \sum_i w_{lj} f(i, j) \]
\[ \text{Eq. 25} \]

to get from the first to the second line we have subtracted the stationary probability \( p_0 (l) = \sum_i w_{lj} p_0 (i) \) on the r.h.s. and integrated both sides. This yields a set of algebraic equations:
\[ p_0 - \epsilon_j = W \cdot f_j \]
\[ \text{Eq. 26} \]

with \( f_j = (f(1, j), f(2, j), \cdots)^T \) and \( \epsilon_j \) is the unit vector in the \( \ell \)-th direction. As in the case of the stationary probability, Eq. 26 alone does not determine \( f_j \) unambiguously but requires an additional condition that can be found by the following consideration:
\[ \sum_i f(i, j) = \int_0^\infty d\tau \sum_i [p(i, t + \tau|j, t) - p_0 (i)] \]
\[ = \int_0^\infty d\tau \ [1 - 1] = 0. \]
\[ \text{Eq. 27} \]

In conclusion, we can determine the auxiliary functions \( f(i, j) \) using Eq. 26 and the additional condition Eq. 27.
Together with the stationary probabilities these determine the noise intensity by:

$$D_t = \sum_i \sum_j x(i) x(j) f(i, j) p_0(j).$$  \hfill (28)

**Reduction of the two-component model to a stochastic IF model: Diffusion approximation of the puff current**

An essential property of Ca$^{2+}$ signaling is its hierarchical organization into blips, puffs, and spikes. In the previous section, we looked at puff statistics determined by the kinetic model of an IP$_3$R cluster. These puffs give rise to a stochastic Ca$^{2+}$ current, the puff current, that feeds into the dynamics of the cytosolic Ca$^{2+}$ concentration and influences associated statistics, for instance, statistics of the ISIs $T_i = t_{i+1} - t_i$ that is the time between two subsequent Ca$^{2+}$ spikes. Calculating ISI statistics from Eq. 2 is difficult because the noise originating from the puff current is a complicated stochastic process, i.e., it does not correspond in general to the simple white Gaussian noise that permits an analytical treatment via the FPE. However, the timescale separation between the puff current and $c_1$ leak current allows for a diffusion approximation in which the stochastic puff current $j_{puff}$ is substituted by a deterministic mean and Gaussian white noise. The resulting Langevin equation can be efficiently numerically integrated and key statistics of $c_1$ and the ISI can be calculated analytically by means of the associated FPE. Discrete-time Markov chains have already been approximated by Langevin equations in the past. To this end, it is usually assumed that the fraction of channels (or clusters in our case) in each state of the Markov chain can be considered as a continuous random variable (see, for instance, (64)). The disadvantage of this method is that the number of channels/clusters must be large compared with the number of states—a condition that cannot be tested experimentally. In contrast, our method relies solely on the observed timescale separation.

To derive the approximate Langevin equation, we first consider Eq. 2 integrated over a short time bin (time window):

$$c_i(t + \Delta t) = c_i(t) - \int_{t}^{t + \Delta t} dt' (c_i(t') - c_0)/\tau + \int_{t}^{t + \Delta t} dt' p\Delta c_{er} \sum_{k=1}^{K} x_k(t')$$

and define:

$$Y(t; \Delta t) = \frac{1}{\Delta t} \int_{t}^{t + \Delta t} dt' \sum_{k=1}^{K} x_k(t').$$

(29)

Here, $Y(t; \Delta t)$ can be regarded as a box-filtered version or moving average of the cluster activity $\sum_k x_k(t)$ and we have explicitly highlighted its parametric dependence on the integration window $\Delta t$. As a result of the filtering, $Y(t; \Delta t)$ is continuous random process with piecewise continuous derivative. The goal is to find a time bin $\Delta t$ so that the following two conditions are met. First, $\Delta t$ must be chosen large enough for $\sum_k x_k$ to experience many transitions. This will ensure that, according to the central limit theorem, $Y(t; \Delta t)$ is Gaussian distributed with mean $\mu_Y$ and variance $\sigma^2_Y$ (calculate in the appendix).

Secondly, $\Delta t$ has to be chosen small enough so that $c_i(t)$ does not change considerably over this time bin. If these two constraints can be met simultaneously, Eq. 29 can be approximated by:

$$c_i(t + \Delta t) \approx c_i(t) - (c_i(t) - c_0) \Delta t/\tau + p\Delta c_{er} Y(t; \Delta t) \Delta t,$$

$$c_i(t) - (c_i(t) - c_0) \Delta t/\tau + p\Delta c_{er} \sigma_Y(\Delta t) v(t) \Delta t,$$

(31)

with the zero mean and unit variance Gaussian random numbers $v(t)$.

Note that the mean $\mu_Y(c_i)$ and more importantly the variance $\sigma^2_Y(c_i)$ are $c_i$ dependent due to the $c_i$ dependence of the opening rate. Eq. 31 has strong similarities to an Euler integration scheme of a Langevin equation. An important difference is that the Gaussian random numbers $v(t)$ in Eq. 31 are not uncorrelated, corresponding to a nonvanishing correlation time $\tau_Y$ of the process $Y(t; \Delta t)$; the correlation time is shown in Fig. 4D. If, however, this correlation time is short compared with the timescale of the $c_1$ leak current, we may assume the Gaussian random numbers to be uncorrelated. In the same limit one can show (see appendix) that the standard deviation $\sigma_Y(\Delta t) \propto 1/\sqrt{\Delta t}$, resulting in the correct scaling of the noise term in the discretized Langevin equation (65) and we obtain for the puff current

$$j_{puff} \approx \mu_{puff}(c_i) + \sqrt{2D_{puff}(c_i)} \xi(t),$$

(32)

so that Eq. 31 approximately corresponds to

$$\dot{c}_i = (c_i - c_0)/\tau + \mu_{puff}(c_i) + \sqrt{2D_{puff}(c_i)} \xi(t),$$

(33)

if $c_i(t) \rightarrow c_T \rightarrow t_i = t$ and $c_i(t) = c_R$. Here, $\mu_{puff} = p\Delta c_{er}\mu_Y$ is the mean puff current, $\xi(t)$ is a zero mean Gaussian white noise with $\langle \xi(t) \xi(t') \rangle = \delta(t - t')$, and $D_{puff} = (p\Delta c_{er})^2 \sigma^2_Y(\Delta t)/2$ is the noise intensity. To summarize, we have first approximated the discontinuous cluster activity $\sum_k x_k(t)$ by a coarse-grained continuous random process $Y(t)$ that is assumed to be Gaussian distributed. In a second step this random process is approximated by a Gaussian white noise, hence assumed to be uncorrelated on the relevant timescale of the $c_i$ dynamics. The resulting
stochastic differential equation with a \( c_1 \)-dependent noise intensity is an equation with multiplicative noise. Such an equation requires an interpretation \( 66 \) the most popular of which are the ones by Ito, by Stratonovich, and by Klimontovich and Hanggi \( 67 \), and it is not immediately clear which one of them, if any, should be used for our model. However, it is known that according to Wong and Zakai \( 68 \), a Langevin equation should be interpreted in the Stratonovich sense if the multiplicative noise is an approximation of a continuous random process that has a piecewise continuous derivative, as it is the case for \( Y(t) \). The corresponding FPE for the Stratonovich-interpreted Eq. 33 is given by:

\[
\begin{align*}
\partial_t p(c_1, t) &= \partial_{c_1} \left[ (c_1 - c_0) / \tau - \mu_{\text{puff}}(c_1) - D'_{\text{puff}}(c_1) / 2 \right. \\
& \hspace{1cm} + \left. \partial_{c_1} D_{\text{puff}}(c_1) \right] p(c_1, t) + r_0 \delta(c_1 - c_R).
\end{align*}
\]

(34)

where \( D'_{\text{puff}}(c_1) / 2 \) is the Stratonovich drift and the prime denotes the derivative with respect to \( c_1 \). The unusual source term \( r_0 \delta(c_1 - c_R) \) accounts for the influx of probability due to the reset of the trajectories that reach the threshold (for the analytical treatment, see the literature from computational neuroscience \( 32,69–71 \)). Eq. 34 is completed by the natural boundary condition \( \lim_{c_1 \to -\infty} p(c_1, t) = 0 \), the absorbing boundary condition at the threshold \( p(c_T, t) = 0 \), and the normalization condition \( \int dc_1 p(c_1, t) = 1 \). Even though we opted above for the Stratonovich interpretation, it is nevertheless interesting to explore the consequence of the interpretation for the ISI statistics which is done below (see Fig. 6 and surrounding discussion). In brief, the choice of the interpretation has only a minor effect on the ISI statistics in the biophysically relevant parameter range.

Regarding the spike train produced by the IF model Eq. 33, we note that irrespective of the specific form of the \( \text{Ca}^{2+} \)-dependent drift and noise terms, the model always generates a renewal process \( 72 \), i.e., a point process with statistically independent intervals between adjacent spikes. This is so because the reset of the \( \text{Ca}^{2+} \) variable \( c_1 \) to a fixed value \( c_R \) at the end of one interval erases any memory about this interval and the uncorrelated noise process \( \xi(t) \), by definition, cannot carry any memory from one ISI to the next either.

**Statistics of the subthreshold cytosolic \( \text{Ca}^{2+} \) and the spike train**

In the previous section we have introduced a one-dimensional Langevin approximation of the two-component model and stated the associated FPE. In particular the latter equation permits the analytical calculation of statistics of interest of the subthreshold \( \text{Ca}^{2+} \) concentration and of the ISIs (see appendix). Here, we compare these analytical results to numerical simulations of the two-component model Eq. 2. We will also give an overview how the spike statistics

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**FIGURE 4** Diffusion approximation of the puff current. For the puff current to be well approximated by a Gaussian white noise, there has to be a time bin \( \Delta t \), so that \( Y(t, \Delta t) \) is 1) Gaussian distributed and 2) shortly correlated compared with every other timescale present in the system. \( (A \) and \( B \) \) Show exemplary time series of the random process \( Y(t, \Delta t) \) together with the probability distribution \( P(Y) \) (histogram) for two different values of the \( \text{Ca}^{2+} \) concentration \( c_1 \) \( (c_1 = c_R \) in \( A \) and \( c_1 = c_T \) in \( B \).\) We also show the effect of different time bins \( \Delta t \) over which the cluster activity \( \sum x_i \) was averaged according to Eq. 30: \( \Delta t = 0.1 \) \((A_1, B_1)\) and \( \Delta t = (A_2, B_2)\). The probability distribution \( P(Y) \) resembles more strongly the desired Gaussian distribution (solid red line) when \( \Delta t \) and \( c_1 \) are larger because then a larger number of transitions in the considered time bin occur. In \( (C \), the deviation of \( P(Y) \) from a Gaussian distribution is quantified by the skewness \( \gamma_Y = ((Y - \langle Y \rangle)^3) / \langle (Y - \langle Y \rangle)^2 \rangle^{3/2} \) of \( P(Y) \), which decreases with increasing \( \Delta t \). \( (D \) Illustrates the timescale separation between the mean ISI {}\( \langle T \rangle \), the timescale of the \( c_1 \) leak current {}\( \tau \), and the correlation time {}\( \tau_Y \) of the random process \( Y(t) \). The latter approaches \( \tau_Y = \Delta t \) for large values of \( \Delta t \) (dotted line). Parameters are chosen according to Fig. 2 and {}\( K = 10 \). To see this figure in color, go online.
depends on the system parameters, specifically the timescale of the leak current \( \tau \), the permeability-like parameter \( p \), and the number of clusters \( K \), i.e., the parameters that are presumably most prone to cell-to-cell variability.

By means of the Langevin approximation of the two-component model two firing regimes can be distinguished. In the mean-driven regime the model crosses the spiking threshold even in the absence of fluctuations and spiking is typically more regular. In the excitable (fluctuation-dependent) threshold even in the absence of fluctuations and spiking is more variable. The two regimes can be distinguished by the deterministic drift term in the Langevin equation, \( f(c) = - (c - c_0)/\tau + \mu_{\text{puff}}(c_i) \), which in the mean-driven regime is positive for all subthreshold \( \text{Ca}^{2+} \) concentration, \( c_i < C_T \), but has a zero for some \( c_i^* < C_T \) in the excitable case. The regimes are separated by the condition that the zero of \( f \) is equal to the threshold, \( f(C_T) = 0 \), which implies \( (C_T - c_0)/\tau = \mu_{\text{puff}}(C_T) \). The fact that the mean puff current depends linearly on the permeability-like parameter \( \mu_{\text{puff}}(c_T) = \rho \Delta c_T K \mu_s \) (with \( \mu_s \) defined in Eq. 22) leads to a simple condition for the critical values of \( \tau \) and \( p \):

\[
\tau p = \frac{c_T - c_0}{\Delta c_T K \mu_s}. \tag{35}
\]

The behavior of the two-component model in the mean-driven and excitable regime is shown in Fig. 5, A and B, respectively. In Fig. 5, A and B we show typical time series of the \( \text{Ca}^{2+} \) concentration and puff current and additionally indicate the corresponding ISI sequences. It can be easily seen that spiking is more regular in the mean-driven regime than in the excitable regime.

In Fig. 5, A and B, the corresponding stationary probability densities \( p_0(c_i) \), obtained from stochastic simulation of the two-component model (histograms), are compared with the theoretical predictions (red line) according to Eq. 61 and demonstrate excellent agreement. In the mean-driven regime the probability is close to uniform (Fig. 5 A1) due to the little variation shown by the drift (cf. inset Fig. 5 A2). In the excitable regime the deterministic drift has a zero, see inset Fig. 5 B2. The system spends more time around the zero, which is reflected in a pronounced maximum of the probability density (Fig. 5 B2). The maximum is not exactly at the zero but at a somewhat smaller concentration due to the interplay between the multiplicative noise and the nonlinear drift term.

In Fig. 5, A3 and B3 we show the resulting ISI density obtained from simulations of the two-component model by histograms. In the mean-driven regime the distribution of the ISIs is strongly peaked (Fig. 5 A3); the sequence of ISIs is rather regular with a moderate CV of \( CV_T = \sqrt{\langle T^2 \rangle/\langle T \rangle} = 0.2 \). The probability density of the ISI is well described by both an inverse Gaussian and gamma distribution:

\[
p_{\text{IG}}(T) = \sqrt{\langle T \rangle/2\pi \sigma^3} \exp \left( - \frac{(T - \langle T \rangle)^2}{2\langle T \rangle \sigma^2} \right), \tag{36}
\]

\[
p_{\text{Gamma}}(T) = \frac{1}{\Gamma(1/\sigma^2) T} \left( \frac{T}{\langle T \rangle} \right)^{1/\sigma^2} \exp \left( - \frac{T}{\langle T \rangle} \right) \tag{37}
\]

where \( \Gamma(a) = \int_0^\infty dt t^{a-1} e^{-t} \) is the gamma function. This densities are fully determined by the mean and CV of the ISI. We use two versions of the formulas: one where we determine \( \langle T \rangle \) and \( CV_T \) from stochastic simulations of the two-component model (dashed line) and another one where we calculate \( \langle T \rangle \) and \( CV_T \) from the Langevin approximation. It turns out that in the mean-driven regime both densities and versions agree very well, the latter indicates that the Langevin equation provides a good approximation.

In the excitable case, the ISI sequence is much more variable with a \( CV_T = 0.8 \) and its probability density (Fig. 5 B3) displays a pronounced skewness. Surprisingly, even in this case the inverse Gaussian Eq. 36 provides a good description when we use the mean and CV obtained from simulation of the two-component model, whereas the mean and CV calculated from Eq. 62 and Eq. 68 leads to a somewhat more peaked distribution. Here, the Langevin approximation does not work so well anymore because in the excitable regime the timescales of the leak and the puff currents are no longer strongly separated.

Going beyond the two specific sets of parameters inspected so far, we show in Fig. 6, A and B the mean and CV of the ISIs obtained from stochastic simulations of the two-component model at values of the timescale \( \tau \) and permeability-like parameter \( p \) varied over two orders of magnitude. Plausibly, the mean ISI (Fig. 6 A) drops systematically with growing \( p \) (which increases the mean and noise intensity of the puff current) and growing \( \tau \) (which decreases the leak current). We also show the boundary between excitable and mean-driven regimes according to Eq. 35 (dashed red line). Because we consider only noise originating from the IP_3R clusters as the sole source of fluctuations, there is also a large chunk of the parameter space at small values of \( p \) and \( \tau \) where no spiking is observed (white area) and the excitable regime attains only a small portion below the bifurcation line. The CV (Fig. 6 B) displays a more complicated dependence on the parameters. It is usually high, close to one, in the excitable regime (73) and drops drastically as soon as we cross the bifurcation line by increasing \( p \) or \( \tau \). In the limit of large \( \tau \), i.e., when the leak current becomes negligible, the CV saturates. In contrast, for large \( p \) the CV keeps increasing. This is presumably due to the increasing noise intensity combined
with the fact that the leak current becomes less important for $p \to \infty$.

We can repeat the same simulations using the Langevin Eq. 33 to obtain the mean and CV, and we can do this not only for the Stratonovich interpretation of this equation, on which our analytical calculations are based, but also for the other two popular interpretations. In Fig. 6, $C_1-C_2$ and $D_1-D_3$ we show the relative differences between mean and CV obtained from stochastic simulation of the two-component model and the Langevin equation in the three interpretations as discussed around Eq. 34. We find that in the biophysically relevant parameter range the three interpretations yield similar results and thus the interpretation of the stochastic differential equation is not a vital issue for our model.

Returning to the two specific sets of parameters, in Fig. 7 we show essential ISI statistics of the model. In Fig. 7, A1 and B1 we plot the firing rate $r_0 = 1/(T)$ as a function of the stimulus amplitude, the relative IP$_3$ concentration $s$ (the arrow indicates the value of $s$ that was used so far and is used in the remaining panels of this figure). Each circle corresponds to a stochastic simulation of the two-component model and is color-coded according to the corresponding CV$_T$ obtained from the same simulation. The theoretical prediction of the firing rate according to Eq. 62 shows excellent agreement with the simulation results in the mean-driven regime (Fig. 7 A1) and displays small deviations in the excitable regime (Fig. 7 B1). We note the similarity of these rate-versus-stimulus curves to the f-I curves (frequency versus input current) used in computational neuroscience (see, e.g., (26,74)).

Next we consider the spike count, the integral of the spike train $z(t) = \sum_t \delta(t - t_i)$:

$$N(t) = \int_0^t dt' z(t'),$$ (38)

and plot in Fig. 7, A2 and B2 an important statistical measure characterizing count variability, namely the Fano factor:

$$F(t) = \frac{\langle \Delta N(t)^2 \rangle}{\langle N(t) \rangle}.$$ (39)

This measure compares the variance to the mean of the spike count as a function of the counting window $t$. For time windows $t$ much smaller than the mean ISI, finding a spike in that window is essentially a Poisson process so that $\lim_{t \to 0} F(t) = 1$ (75). At intermediate times, the Fano factor decreases linearly (not very apparent due to the used logarithmic scale) up to a characteristic time which is roughly the mean ISI. For large values of $t$ and

---

**FIGURE 5** Two-component model in the mean-driven and excitable regime. (A and B) The model is in the mean-driven and excitable regime, respectively. (A1 and B1) Show the subthreshold dynamics of $c_1$ and the puff current $j_{\text{puff}}$. Already with the naked eye it can be seen that the corresponding ISI sequence marked by black arrows is more regular in (A1) than in (B1). (A2 and B2) We compare the corresponding subthreshold probability densities $p_0(c_1)$ obtained from stochastic simulations of the two-component model (histograms) to the theoretical prediction according to Eq. 61 (red lines). The insets in (A2) and (B2) show the drift $f(c_1)$ that is always positive in the mean-driven regime (A2) but exhibits a zero at $c_1^*$ in the excitable regime (B2). (A3 and B3) The ISI densities determined from numerical simulations (histograms) are compared with inverse Gaussian and gamma distributions (red lines) as indicated by the red text. Both distributions are uniquely determined by the mean $\langle T \rangle$ and CV CV$_T$ that have been taken either directly from stochastic simulations of the two-component model (dashed red lines) or calculated according to Eq. 62 and Eq. 69 (dotted red lines). In the mean-driven regime all four variants are in excellent agreement with the ISI density obtained from the two-component model. In the excitable regime the inverse Gaussian provides a better estimate than the gamma distribution to the ISI density obtained from stochastic simulations of the two-component model. In addition, mean and CV obtained from the two-component model and the Langevin approximation begin to differ so that the distributions (dashed and dotted red lines) disagree. Parameters: $K = 10$, (A): $\tau = 5, p = 1.5 \times 10^{-2}$, (B): $\tau = 1, p = 6 \times 10^{-2}$. To see this figure in color, go online.
assuming that the spike train is renewal, as we have argued previously, the Fano factor saturates at the squared CV limit
\[ \lim_{\tau \to \infty} CV^2 = \frac{CV^2}{\tau}. \]
which is confirmed in the figure as well.

Finally, we turn to the power spectrum of the spike train \( z(t) \), defined by:

\[
S(f) = \lim_{T \to \infty} \frac{\langle |\tilde{z}(f)|^2 \rangle}{T},
\]

\[
\tilde{z}(f) = \int_0^T dt \, z(t)e^{i2\pi ft},
\]

where is the Fourier transform. In the renewal case the spike train is fully determined by the ISI density and the power spectrum can be related to its Fourier transform \( \tilde{p}_{\text{ISI}}(f) \) as follows (76):

\[
S_{\text{renew}}(f) = \frac{1}{\langle T \rangle} \frac{1 - |\tilde{p}_{\text{ISI}}(f)|^2}{1 - |\tilde{p}_{\text{ISI}}(f)|^2},
\]

\[
\tilde{p}_{\text{ISI}}(f) = \int_0^\infty dt \, p_{\text{ISI}}(t)e^{i2\pi ft}.
\]

The Fourier transformed of the inverse Gaussian distribution Eq. 36 is given by:

\[
\tilde{p}_{\text{IG}}(f) = \exp \left( \frac{1 - \sqrt{1 - 4\pi f \langle T \rangle CV^2}}{CV^2} \right).
\]

In Fig. 7, A and B, we show the power spectrum directly calculated from the spike train of the two-component model according to Eq. 40 (blue lines) and compare it to the power spectrum of an inverse Gaussian ISI density according to Eq. 41 and Eq. 42. The more regular spiking in the
mean-driven regime becomes apparent by a pronounced peak of the power spectrum at the firing rate \( f = r_0 \). In the excitable case, the spectrum is rather flat close to the power spectrum of a Poisson process (which would be perfectly flat). In both cases the spike-train power spectrum, as known from point process theory, saturates at the firing rate for \( f \to \infty \) and attains the value \( r_0 CV_T^2 \) for \( f \to 0 \); these limits are also confirmed in the plot.

**Spike train statistics of stimulated HEK cells**

Here, we compare ISI statistics measured from HEK cells under constant stimulation, as described in (10,14), to those of an appropriately fitted two-component model. Regarding the experimental data, because long traces of \( \text{Ca}^{2+} \) signals are notoriously hard to measure, it is difficult to achieve ISI sample sizes that suffice to meaningfully determine the ISI density, Fano factor and power spectrum. To overcome this problem we pursue a similar strategy as suggested by (77) and rescale the time for each spike train. Specifically, we normalize and pool the corresponding ISI sequences, similar to the one shown in Fig. 1 A, from 29 different HEK cells as follows. In a first step we select from the available data only the ISI sequences that become stationary according to visual inspection (29/36 sequences). In a second step we perform for the selected sequences a fit of the ISIs \( T_i \) by an exponential function, \( T_\infty = (T_\infty - T_0) \exp(-i/n_\tau) \), and truncate the sequences by the first \( 1.5n_\tau \) ISIs (rounded up, yielding on average 6 transient out of 20 total intervals). In a third step we normalize the sequences by dividing each interval by the sequence’s mean. Finally, we concatenate the normalized sequences to obtain a single long sequence of ISIs that does not distinguish between different cells anymore and from which we calculate the statistics of interest. It should be clear that both the mean of this ISI sequence and the firing rate of the corresponding spike train are equal to one.

To find the parameter set for the two-component model we require that the model’s ISI sequence should have the same mean value \( (T_{\text{exp}}) \approx 160 \text{ s} \) and \( CV_{T_{\text{exp}}} \approx 0.15 \) as the experimental data before and after rescaling, respectively. As our model has many parameters, we fix cluster parameters according to the table in Fig. 2 and use \( K = 10 \). To find the parameter set for the two-component model we require that the model’s ISI sequence should have the same mean value \( (T_{\text{exp}}) \approx 160 \text{ s} \) and \( CV_{T_{\text{exp}}} \approx 0.15 \) as the experimental data before and after rescaling, respectively. As our model has many parameters, we fix cluster parameters according to the table in Fig. 2 and use \( K = 10 \). To find the parameter set for the two-component model we require that the model’s ISI sequence should have the same mean value \( (T_{\text{exp}}) \approx 160 \text{ s} \) and \( CV_{T_{\text{exp}}} \approx 0.15 \) as the experimental data before and after rescaling, respectively. As our model has many parameters, we fix cluster parameters according to the table in Fig. 2 and use \( K = 10 \).
In Fig. 8B the ISI distribution of the rescaled and concatenated spike train obtained from stimulated HEK cells (red histogram) is compared with the rescaled ISI distribution obtained from stochastic simulations of the two-component model (black line). Given the limited amount and temporal accuracy of the experimental data, the two probability densities agree well with each other. The Fano factors for the experimental (probability densities agree well with each other. The and temporal accuracy of the experimental data, the two concatenated spike train obtained from stimulated HEK component model (cf. Fig. 7). Finally, in Fig. 8C we present the power spectrum of the experimental spike train (red line) and compare it to the spectrum of the model (black line). Again, the two spectra show excellent agreement in terms of the low frequency limit, height of the peak at $f = 1/(T)$ as well as the high-frequency limit. The latter is not surprising because for $f \to \infty$ the power spectrum always saturates at the firing rate $\lim_{f \to \infty} S(f) = r_0$. The low-frequency limit of the power spectrum is informative because it is related to the firing rate and CV in a simple manner $S(f = 0) = r_0 CV^2$ if the spike train is renewal. The fact that the power spectrum indeed saturates at this limit thus indicates that the spike train is renewal and that the ISIs are uncorrelated. This is somewhat unexpected because the experimental sequences of ISIs often show long transients corresponding to a strong cumulative refractoriness that builds up over many intervals as seen in Fig. 1A. Naively, one would expect that such transients go along with a slow process that leads to correlations between intervals, that in turn affect the low-frequency limit of the power spectrum as follows (79):

$$S_{\text{nonnew}}(f = 0) = r_0 CV^2 \left(1 + 2 \sum_k \rho_k\right).$$

Here, $\rho_k = \text{Covar}(T_i, T_{i+k}) / \text{Var}(T_i)$ are the serial correlation coefficients that quantify correlations between ISIs lagged by an integer value $k$. Indeed, it was shown previously by (36) that stimulated HEK cells exhibit no statistically significant interval correlations. We address and resolve this apparent contradiction of a strong cumulative refractory period without significant interval correlations in a follow-up paper, where we extend the renewal model discussed here by an equation describing the depletion and slow refilling of the ER.

**Dependence of the statistics on cluster number and cluster dynamics**

So far, we have fixed the parameters of the clusters, i.e., their number, the rates etc. Here, we consider the effects of the mean and variance of the IPI and the number of clusters $K$ on the mean and CV of the ISI.

The mean and variability of the IPIs in the cluster model are largely controlled by $\lambda_{\text{ref}}$, the transition rate between the refractory states. Increasing this parameter makes the IPIs shorter on average because the dwell time in the refractory states is shortened. The IPI for larger $\lambda_{\text{ref}}$ will also be less regular because it is then dominated by the single exponentially distributed transition that corresponds to the opening of the cluster. What is the effect of an increase of $\lambda_{\text{ref}}$ on the mean and CV of the ISI? In Fig. 9, A and B the mean ISI and CV both display a systematic decrease with the refractory rate. Hence, paradoxically, a more regular IPI leads to a more irregular ISI.
An explanation for the observed behavior can be given in terms of the mean puff current (Fig. 9, A2 and C) and the noise intensity (Fig. 9, B2 and D) with respect to different values of the refractory rate \( \lambda_{\text{ref}} \). Let us first consider the case of a decreasing refractory rate corresponding to a more regular IPI. This leads to an overall decrease of both the mean puff current and noise intensity as \( \lambda_{\text{ref}} \) increases. Consequently, the mean ISI decreases stems from a decrease of the mean and noise intensity of the puff current. Likewise the transition to the excitable regime stems from a decrease of the mean puff current. The saturation of the CV in (B) is reasonable if the model operates far from the bifurcation in the mean-driven regime and both the drift and noise intensity are scaled by similar factor as we argue in the main text. Parameters: \( \tau = 5, p = 1.5 \times 10^{-2}, K = 10 \).

To see this figure in color, go online.

Another dependence of interest concerns the number of clusters \( K \), which may differ from cell to cell. A simple effect of increasing \( K \) is that the mean puff current and also the noise intensity increases in proportion to \( K \), which is a consequence of the assumed statistical independence of the clusters. Both these increases will lead to shorter ISIs, as seen in Fig. 10 A1. Again (comparable to the increase in \( \lambda_{\text{ref}} \) discussed above) the effect on the variability of the ISI is less clear because the increase in the mean and the increase in the noise intensity have opposing effects on the CV, at least in the mean-driven regime. Inline with the above argument, in Fig. 10 A2 we see only small changes in the CV for a growing number of cluster. We note that for the parameters used here, we do not reach the excitable regime (again indicated by the gray area in

In the opposite case of an increasing refractory rate, and hence stronger and more variable drive of the IF dynamics, the effect of this on the CV is less clear. In terms of the ISI variability a stronger drive results in more regular spiking, i.e., a smaller CV of the ISI. However, the simultaneous increase of the noise intensity has the opposite effect on the ISI variability, and, hence, it is not clear which of the two will win. However, we can resort to a simpler case to develop an intuition. If both the drift and the diffusion coefficient would not depend on \( c_i \) and would be scaled by the same factor (as is roughly the case [cf. Fig. 9, C and D], the CV would not change at all—this is the case of the so-called perfect IF model in neuroscience [see Eq. 20 in (78)] and further references therein). Indeed, if we start from our standard value at \( \lambda_{\text{ref}} = 20 \text{ s}^{-1} \) (indicated by the cross in Fig. 9, A and B) and increase the refractory rate further, the CV does not change much in accordance to this reasoning. In conclusion, the more regular the IPI (the smaller \( \lambda_{\text{ref}} \) the less regular the ISI.
Fig. 10, A1 and A2) without entirely suppressing spiking. If we would reach the excitable regime (by changing other parameters), we would encounter much stronger increases in the CV comparable to what we have seen in Fig. 9 B1 for small values of λ_{ref}.

Finally, we turn to a situation in which the cluster density is preserved while we increase K (Fig. 10, B1 and B2). This roughly corresponds to an increase in cell size which increases the number of clusters but decreases the change of the Ca^{2+} concentration such that the mean puff current is conserved. Accordingly, we study now a scaling in which pK = const.; the ISI statistics for this case are shown in Fig. 10, B1 and B2. Since the mean puff current does not change by construction, we see only very little change in the mean ISI in this case (Fig. 10 B1). The dominating change in this scaling is a reduction of the noise intensity by 1/K. This, however, has only little effect on the CV (Fig. 10 B2) because the relative change of the noise intensity remains small in the considered range of K. Because the system operates in the strongly mean-driven regime, again the scaling argument for the CV of an IF model with constant drift and constant diffusion (78) is meaningful; indeed the observed curve can be perfectly fitted by CV_{T} \propto 1/\sqrt{K} (not shown).

**Extension of the model by an additional fast Ca^{2+} buffer: Effects on the spiking train statistics**

An important element in Ca^{2+} signaling that we have not taken into account so far, are buffer proteins that may bind up to 99% of the free Ca^{2+} both in the cytosol and ER (80,81). Here, we extend our model by an additional Ca^{2+} buffer and discuss how the mean and CV of the ISI are affected. The reaction scheme for the buffer B reads:

\[
Ca^{2+} + B \xrightarrow{k_{-}^{-}} CaB,
\]

where \(k_{^+}\) and \(k_{^-}\) are rate constants (often assumed to be large corresponding to a fast buffer) and the dissociation constant is given by \(K^{*} = k_{^-}/k_{^+}\). The differential equations for the cytosolic Ca^{2+} and the fast buffer concentration can be formulated as follows:

\[
\begin{align*}
\dot{c}_{i} &= - (c_{i} - c_{0})/\tau + j_{puff}(c_{i}) - k_{^+}c_{i}(b_{T} - c_{b}) + k_{^-}c_{b} \\
\dot{c}_{b} &= k_{^-}c_{b}(b_{T} - c_{b}) - k_{^-}c_{b} \\
\text{if } c_{i}(t) &= c_{T} \rightarrow t_{i} = t \text{ and } c_{i}(t) = c_{R}; c_{b}(t) = c_{0}(c_{R})
\end{align*}
\]

(45)

where \(b_{T} = b + c_{b}\) is the total buffer concentration given by the sum of the free and Ca^{2+}-bound buffer concentration \(b\) and \(c_{b}\), respectively; we assume \(b_{T}\) to be constant in time. In an IF framework a fast buffer, that is always in equilibrium with the cytosolic Ca^{2+} concentration, requires an additional reset rule for the bound buffer concentration \(c_{b}\). This condition is given in the last line of Eq. 45 and states that \(c_{b}\) is reset to the equilibrium value, \(c_{0}(c_{R}) = c_{b_{T}}/(K^{*} + c_{i})\), taken at the reset \(c_{i} = c_{R}\). This reset rule ensures that \(c_{b}\) remains in equilibrium even after the infinitely fast reset of \(c_{i}\). According to (82) a fast buffer can be eliminated and affects the dynamics of \(c_{i}\) only through a \((c_{i}\) dependent) factor \(\beta\):

\[
\begin{align*}
dc_{i}/dt &= \beta[- (c_{i} - c_{0})/\tau + j_{puff}(c_{i})], \\
\beta &= (1 + K^{*} b_{T}/(K^{*} + c_{i})^2)^{-1}.
\end{align*}
\]

(46)

Assuming that the \(c_{i}\) dependence of \(\beta\) is weak \((K^{*} \gg c_{i})\) one could wrongly conclude that adding a fast Ca^{2+} buffer simply leads to a rescaling of the time, hence affects the mean but not the CV of the ISIs. However, this is not true. To make this clear consider the Langevin approximation of Eq. 46 and substitute \(t = \tilde{t}/\beta\). Considering the dynamics with respect to this new time leads to a deterministic drift that is independent of \(\beta\) but a noise intensity that is not:

\[
\begin{align*}
dc_{i}/d\tilde{t} &= f(c_{i}) + \sqrt{2\beta D_{puff}(c_{i})\xi(\tilde{t})},
\end{align*}
\]

(47)

due to the scaling properties of the white noise \(\sqrt{\beta \xi(\tilde{t})} = \xi(\tilde{t}/\beta)\). (Rescaling the time \(t = \tilde{t}/\beta\) of a white noise process affects its intensity because the autocorrelation function should be preserved, i.e., \(2D\delta(t - t') = 2D'\delta(\tilde{t} - \tilde{t}')\). Using the scaling property of the Dirac delta function \(\delta(\tilde{t} - \tilde{t}')/\beta) = |\beta|\delta(\tilde{t} - \tilde{t}')\) allows to relate the two intensities \(D' = \beta D\) so that one finds \(\sqrt{\beta \xi(\tilde{t})} = \xi(\tilde{t}/\beta)\).

In the mean-driven regime the average ISI depends strongly on the drift but only weakly on the noise intensity. Because in the rescaled time the drift does not depend on \(\beta\), this implies that average ISI in the rescaled dynamics is also independent of \(\beta\). Going back to the original time, intervals scale linearly with \(1/\beta\) as time itself:

\[
\langle T \rangle \approx \langle T_{0} \rangle / \beta \approx \langle T_{0} \rangle (1 + b_{T} / K^{*})
\]

(48)

where \(\langle T_{0} \rangle\) refers to the mean ISI measured in units of \(\tilde{t}\) or, equivalently, to the mean ISI measured in units of \(t\) but without a buffer, \(b_{T} = 0\). If we further assume that \(b_{T}/K^{*} \gg 1\), Eq. 48 implies an approximately linear relationship between \(\langle T \rangle\) and the total buffer concentration \(b_{T}\). In Fig. 11 A1 we demonstrate this linear relation, Eq. 48 (red line), by means of stochastic simulations of the two-component model with an additional buffer according to Eq. 45 (circles). We also compare this to the Langevin approximation of the same model version, i.e., Eq. 45 where the puff current is substituted according to Eq. 32 (blue line). The two stochastic simulation results agree well with each other; however, they display a slightly slower but still linear increase compared with the simple estimate Eq. 48.
The effect of a fast buffer on the CV is more complicated. However, when the system is in the mean-driven regime and the noise intensity is not too strong, the influence of the buffer concentration on the variance of the ISIs can be estimated according to (83):

\[
\langle \Delta T^2 \rangle = 2\beta \int_{c_1}^{c_2} dc_i D_{\text{puff}}(c_i)/f(c_i)^3,
\]

(49)

where we have again assumed that \( \beta \) is independent of \( c_i \).

The CV is then given by:

\[
CV_T = \frac{\langle \Delta T^2 \rangle / \beta}{(T_0)/\beta} \approx \sqrt{CV_{T,0}} / \sqrt{1 + b_T/K^*},
\]

(50)

where \( CV_{T,0} \) refers to the CV without a buffer. The CV thus exhibits a simple square-root relation with respect to the total buffer concentration that is confirmed in Fig. 11 A2.

In Fig. 11, B1 and B2 we consider a scenario where spiking is fluctuation driven (excitable regime) and the mean interval therefore depends more strongly on the noise intensity and in turn buffer concentration. In particular, the mean interval obtained from stochastic simulations of the two-component model with a buffer grows faster than linear (cf. circles in Fig. 11 B1). This is so because in the excitatory regime the passage to the threshold is facilitated by noise—in addition to diminishing the drift we now also diminish the intensity of the noise by increasing \( b_T \), which leads to a superlinear increase of the mean interval with \( b_T \). Interestingly, for the Langevin approximation this superlinear growth is less pronounced, which is in line with the fact that this approximation works less well in the excitable regime. The variability of the ISIs also drops with \( b_T \); apparently we are here far from the weak-noise limit where the CV can decrease upon a reduction of the noise intensity (we are on the r.h.s. of the coherence-resonance minimum, see (73)).

**SUMMARY AND DISCUSSION**

This study exploited the similarities between stochastic spike generation in neural dynamics and in \( \text{Ca}^{2+} \) dynamics to develop a phenomenological but biophysically grounded model of \( \text{Ca}^{2+} \) spiking and used established methods from computational neuroscience to calculate spike train statistics analytically. The main source of variability is the \( \text{Ca}^{2+} \) noise generated by the stochastic opening of clusters of IP3R channels in the ER membrane leading to stochastic sequence of \( \text{Ca}^{2+} \) release puffs. This cluster noise is similar to the ion channel noise in the membrane potential dynamics of neurons and can be treated mathematically in a similar way, i.e., by a diffusion approximation. Furthermore, for a simplified description of intracellular \( \text{Ca}^{2+} \) spiking, we have adopted the IF framework of computational neuroscience. Our resulting two-component model captures the punctuated release of \( \text{Ca}^{2+} \) from IP3Rs on the one hand and the dynamics of the cytosolic \( \text{Ca}^{2+} \) on the other hand. It can reproduce the spike statistics of HEK cells, ranging from Fano factors to spike train power spectra quantitatively (see Fig. 8).

Specifically, we suggested a phenomenological puff model which meets a variety of experimentally observed puff characteristics. Five observations have been used to design the model: 1) puffs occur randomly (see Yao et al. (84)); 2) the amplitude is uniformly distributed over the number of channels in the cluster (see Dickinson and Parker (50)); 3) dwell times in a cluster state with a given number of open channels are rather independent of that number (see Wiltgen et al. (52)); 4) IPIs may exhibit a refractory period (see Thurley et al. (45)), and 5) the approximate values of the IPI’s mean and refractory period (see Thurley et al. (45)). For the resulting Markov model of the cluster activity we provide expressions for the mean (Eq. 18a) and CV (Eq. 18c) of the IPI as well as the mean (Eq. 13) and CV (Eq. 15) of the puff strength. The CV of the IPI at the resting \( \text{Ca}^{2+} \) concentration resulting from this model is close to 1 with parameters chosen according to Fig. 2, and in agreement with low stimulation results for HEK cells (45), which is our standard example here. SHSY-5Y cells exhibit a CV of 0.68 at low and of 0.42 at high stimulation (45), which can also be modeled by adjusting the cluster parameters accordingly (Eq. 18c admits arbitrarily low CVs if we add a sufficient number of refractory states; it holds specifically that \( \lim_{\alpha \to 0} CV_I = 0 \), cf. Eq. 18c).
Puff activity is one of the currents contributing to the global Ca\(^{2+}\) dynamics. Here, we have shown that because of the timescale separation between the puff dynamics and the cytosolic Ca\(^{2+}\) dynamics the puff current can be well approximated by a white Gaussian noise with a Ca\(^{2+}\)-dependent mean and Ca\(^{2+}\)-dependent noise intensity, functions, which can be calculated from the transition rate matrix according to Eq. 22 and Eq. 28, respectively. This diffusion approximation provides a simple method to determine the Langevin equation corresponding to a given channel cluster model. That might also find applications to models of cardiomyocytes and their Ca\(^{2+}\) release units.

The derived Langevin approximation for the dynamics of the cytosolic Ca\(^{2+}\) concentration permits stochastic simulations that are by orders of magnitude faster than the simulation of the full two-component model. It furthermore allows to distinguish in a simple manner the mean-driven and the excitable regimes. Moreover, in this approximation, different ISI statistics can be calculated analytically, i.e., we can find quadrature expressions for the mean and variance of the ISI and, at least in the mean-driven regime, also simple estimates for the ISI density and spike-train power spectrum. We have also shown that the interpretation of the stochastic differential equation with multiplicative noise (Ito-Stratonovich dilemma (60,67)) is not a major issue for the biophysically relevant parameter range.

Beside validating our approximation we also explored the dependence of the ISI statistics on the biophysical parameters of the cell (the time constant of the leak current \(\tau\) and permeability-like parameter \(p\)) and the clusters (their number \(K\) and refractory rate \(\gamma_{\text{ref}}\)). We have shown that the model in either the mean-driven or excitable regimes can reproduce experimentally measured CV values which range from 0.2 in Vaspressin-stimulated Hepatocytes to 1.0 in spontaneously spiking Astrocytes (10,14,85–87). As already mentioned above, we demonstrated that our model can generate spike trains with statistics highly similar to those of stimulated HEK cells.

Finally, motivated by the experimental results by Skupin et al. (10) we also investigated the effect of incorporating a fast Ca\(^{2+}\) buffer in our model. Here, we find an approximately linear scaling of the mean ISI with the total buffer concentration and in particular the scaling \((T) \propto 1 + b_T/K^+\) in the mean-driven regime, i.e., the model reproduces the experimentally observed increase of the mean ISI with increasing buffer concentration (10). Note that the total buffer concentration \(b_T\) takes into account Ca\(^{2+}\) binding proteins that are naturally present in the cell and hence does not directly correspond to the concentration of the added buffer. Another remarkable experimental finding by Skupin and Falcke (88) was that the CV is largely independent of the concentration of the added buffer. This feature is consistent with our model if it operates in the excitable regime close to the bifurcation Fig. 11 \(B_2\) and we include an offset in the buffer concentration.

As an outlook we note that the CV of experimental spike trains exhibits robustness against cell-to-cell variability. Parameters describing cell variability are the number of clusters and variability of the strength of cluster-to-cluster coupling due to the variable geometry of the cluster array. We have shown that, at least in a scenario with fixed cluster density, our model’s CV is largely independent of the number of clusters. Additional robustness of the CV is likely to arise from a slow recovery from negative feedback which terminated the previous spike, i.e., spike generation happens in a transient (20,89). This transient is assumed to provide the CV robustness properties. We expect that adding such a transient to the Langevin formulation would entail the observed robustness properties. This is studied in a follow-up paper to the study presented here.

Appendix

Mean and variance of the surrogate random process \(Y(i)\)

Here, we relate the mean \(\mu_Y\) and variance \(\sigma_Y^2\) of the coarse-grained random process \(Y(i)\) defined by Eq. 30 to the mean \(\mu_x\) and noise intensity \(D_x\) of the single cluster \(x(t)\) under the assumption that \(c_i\) changes slowly. The means can be readily related to one another using that the mean of a sum of random numbers is the sum of their means. This yields the expression:

\[
\mu_Y = \langle Y(t; \Delta t) \rangle = \frac{1}{\Delta t} \int_t^{t + \Delta t} dt_1 \left\langle \sum_{k} x_k(t_1) \right\rangle
\]

\[
= \frac{1}{\Delta t} \int_t^{t + \Delta t} dt_1 K \langle x(t_1) \rangle
\]

\[
= K \mu_x,
\]

that holds irrespective of any timescale separation.

Relating the variance to the noise intensity of a single cluster is more complicated because it requires that the correlation function of a single cluster decays sufficiently fast. The variance of \(Y(t)\) is given by:

\[
\sigma_Y^2 = \langle \Delta Y(t; \Delta t)^2 \rangle
\]

\[
= \frac{1}{\Delta t} \int_t^{t + \Delta t} dt_1 dt_2 \sum_{k_1} \sum_{k_2} \langle \Delta x_{k_1}(t_1) \Delta x_{k_2}(t_2) \rangle,
\]

\[
= \frac{1}{\Delta t} \int_t^{t + \Delta t} dt_1 dt_2 \left[ \sum_{k_1} \sum_{k_2=1}^{K} \langle \Delta x_{k_1}(t_1) \Delta x_{k_2}(t_2) \rangle
\]

\[
+ \sum_{k_1 \neq k_2} \langle \Delta x_{k_1}(t_1) \Delta x_{k_2}(t_2) \rangle \right],
\]

\[
= K \frac{1}{\Delta t} \int_t^{t + \Delta t} dt_1 dt_2 C_{xx}(t_2 - t_1).
\]
To get from the second to the third line we have 1) used that the clusters are independent of one another so that the covariance $\langle \Delta x_c(t_1) \Delta x_c(t_2) \rangle$ vanishes and 2) introduced the autocorrelation function $C_{xx}(t_2 - t_1) = \langle \Delta x_c(t_2) \Delta x_c(t_1) \rangle$ with $\Delta x = x - \langle x \rangle$. This expression can be further simplified (very similar to the derivation of the Kubo relation (90)) using that the autocorrelation only depends on the difference $\tau = t_2 - t_1$ and is symmetric $C_{xx}(\tau) = C_{xx}(-\tau)$:

$$
\sigma_y^2 = \frac{K}{\Delta t} \int_0^{\tau+\Delta t} dt_1 dt_2 C_{xx}(t_2 - t_1)
= \frac{2K}{\Delta t} \int_0^{\Delta t} d\tau C_{xx}(\tau)(1 - \tau/\Delta t).
$$

(53)

Hence, the autocorrelation function of $x(t)$ determines the variance of the surrogate random process $Y(t)$. This expression can be related to the noise intensity of the puff current through a single cluster:

$$
D_x = \int_0^{\infty} d\tau \ C_{xx}(\tau)
$$

(54)

if, in line with our initial assumption, the correlation time $\tau_y$ is assumed to be small compared with the time-window $\Delta t$. In this case the term $\tau/\Delta t$ in Eq. 53 can be neglected so that we find:

$$
\sigma_y^2 = \frac{2KD_x}{\Delta t}.
$$

(55)

**Stationary probability density and firing rate**

The derived Langevin equation Eq. 33 can be used to calculate the stationary probability density function $p_0(c_i)$ and the firing rate $r_0 = 1/(T)$. To this end, we need to establish the associated FPE (32,69–71) that was already given in the main part and is repeated here for convenience:

$$
\partial_t p(c_i,t) = \partial_c [ -f(c_i) - D'(c_i)/2 + \partial_c D(c_i)]p(c_i,t) + r_0 \Theta(c_i - c_R),
$$

(56)

with the drift term $f(c_i) = -(c_i - c_0)/\tau + \mu_{\text{puff}}(c_i)$ and the noise-induced Stratonovich drift $D'(c_i)/2$. The FPE is completed by the natural boundary condition $p(c_i \to -\infty, t) = 0$, absorbing boundary condition $p(c_T, t) = 0$ and normalization $\int dc_i p(c_i, t) = 1$. We first calculate the stationary firing rate $r_0$ by means of the stationary probability density function $p_0(c_i)$ of Eq. 56:

$$
0 = \partial_c [ -f(c_i) - D'(c_i)/2 + \partial_c D(c_i)]p_0(c_i) + r_0 \Theta(c_i - c_R),
$$

(57)

where we have dropped the time derivative because we are interested in the stationary case and integrated once with respect to $c_i$. To solve this equation we introduce two auxiliary functions $L(c_i) = D(c_i)p_0(c_i)$ and define $g(c_i) = f(c_i) + D'(c_i)/2$ and obtain:

$$
\partial_t L(c_i) = \frac{g(c_i)}{D(c_i)}L(c_i) - r_0 \Theta(c_i - c_R),
$$

(58)

the homogeneous solution $L_h(c_i)$ to this equation is readily obtained:

$$
L_h(c_i) \propto \exp \left( \int_{c_R}^{c_i} dy \frac{g(y)}{D(y)} \right).\quad
$$

(59)

The method of variation of parameters yields the full solution:

$$
L(c_i) = r_0 e^{h(c_i)} \int_{c_R}^{c_i} dx \ e^{-h(x)} \Theta(x - c_R),
$$

(60)

$$
h(x) = \int_{c_R}^{x} dy \ \frac{g(y)}{D(y)}.\quad
$$

(61)

Finally, we substitute the probability density $p_0(c_i)$ again and find:

$$
P_0(c_i) = r_0 e^{h(c_i)} \int_{c_R}^{c_i} dx \ e^{-h(x)} \Theta(x - c_R)\quad
$$

(62)

Note that the desired firing rate $r_0$ can be obtained by means of the normalization condition, put differently we can integrate over $c_i$ from $-\infty$ to and obtain:

$$
r_0 = \left( \int_{c_R}^{c_T} dc_i \ e^{-h(c_i)} \int_{-\infty}^{c_i} dx \ \frac{e^{h(x)}}{D(x)} \right)^{-1}\quad
$$

(63)

For this expression we have used that the boundaries of the integral can be interchanged as follows:

$$
\int_{-\infty}^{c_T} dx_1 \int_{c_R}^{c_T} dx_2 \ f(x_1, x_2) \theta(x_2 - x_R)
= \int_{c_R}^{c_T} dx_2 \int_{-\infty}^{c_T} dx_1 \ f(x_1, x_2).\quad
$$

(64)

**Coefficient of variation**

The CV $CV_T^2 = \langle \Delta T^2 \rangle / \langle T \rangle^2$ can be calculated by considering the ISI $T$ as a first-passage time (FPT). Put differently, we consider a “particle” governed by Eq. 33 with $c_T(T = 0) = c_0$ and ask what the distribution of times is at which it will first cross the threshold $c_T$. This is the so-called FPT density $p_{\text{FPT}}(T)$ that itself is usually not analytical accessible but its moments $\langle T^n \rangle$ are (59). To calculate the moments, we establish the respective FPE (see, e.g., (91)):

$$
\partial_T p(c_i, T) = \partial_c [ -f(c_i) - D'(c_i)/2 + \partial_c D(c_i)]p(c_i, T)
= - \partial_c j(c_i, T),
$$

(64)
with the probability current $j(c, T)$. The boundary and initial conditions are given by:

$$p(-\infty, T) = p(c_r, T) = 0, \quad p(c, 0) = \delta(c - c_0). \quad (65)$$

Note that in contrast to Eq. 56 this FPE does not possess any source terms that correspond to the reset rule of Eq. 33. This is so because for the first-passage time, trajectories that have crossed the threshold are not reset but absorbed. An important insight is that the FPT density is given by the probability current evaluated at the threshold:

$$p_{FP}(T) = j(c_r, T). \quad (66)$$

Calculating the moments of FPT density is, in the one-component case, a standard problem in the theory of stochastic processes (59). Here, we do not present the full derivation but refer to (91) and specifically to the hierarchy of differential equations that determines the moments:

$$-nJ_{n-1}(c) = -g(c)J_n'(c) + \partial_c D(c)J_n''(c), n > 0. \quad (67)$$

Here, $J_n(c_r)$ are exactly the moments of the FPT density and $J_0(c_1) = \Theta(c_1 - c_0)$. The generalization to the case of multiplicative noise is straightforward. The solution to the second moment is given by:

$$\langle T^2 \rangle = 2 \int_{c_r}^{c} dx_1 e^{-h(x)} \int_{-\infty}^{x_1} dx_3 \frac{\phi(x_3)}{D(x_3)} \times \int_{c_r}^{x_3} dx_2 e^{-h(x_2)} \int_{-\infty}^{x_2} dx_1 \frac{\phi(x_1)}{D(x_1)} \langle T^2 \rangle \quad (68)$$

with $h(x)$ as in Eq. 60. The evaluation of these four nested integrals is challenging, even numerically. Luckily, in case of the variance the expression can be simplified greatly to contain only two nested integrals (cf. (70)):

$$\langle \Delta T^2 \rangle = 2 \int_{-\infty}^{c} dx_3 e^{-h(x_3)} \left( \int_{x_3}^{\infty} dx_2 \frac{\phi(x_2)}{D(x_2)} \right)^2 \times \int_{-\infty}^{c} dx_1 e^{-h(x_1)} \Theta(x_1 - c_R). \quad (69)$$

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: Methodology, software, investigation, data curation, writing, and visualization, L.R.; conceptualization, methodology, validation, investigation, writing, supervision, and project administration, B.L.; conceptualization, writing – review & editing, supervision, and project administration, M.F. All authors reviewed the results and approved the final version of the manuscript.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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