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A solution for non-constant mean open time in homogeneous Markov models

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Abstract

The $InsP_3$ receptor is a ligand-gated calcium channel that is modulated both by $InsP_3$ and Ca^{2+}. Recent experiments have shown that the mean open time of the channel is not a monotonic function of $[Ca^{+2}]$. In this work, we propose a solution for this type of behavior in a general framework.

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1. Introduction

Ion channels are proteins that can change conformation depending on various factors. In their open conformation, they allow the passage of ion currents through an usually very narrow pore that acts as a selectivity filter. Thus, ion channels are highly selective, letting the passage of very specific ions. There is a large body of work on modeling ion channels, in particular, on modeling the kinetics of openings and closings. Experimentally, the ion current through a single channel can be measured as a function of time (usually using the "patch-clamp technique"). From a mathematical point of view, these series can be thought of as sequences of zeros (closed states) and ones (open states). Different series may correspond to different stimuli that can be obtained by either changing the voltage across the membrane where the channels are inserted (as it is usually the case for voltage-gated channels) or changing the concentration of an

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agonist, in the case of ligand-gated channels. Among calcium channels, the best-studied ligand-gated ones are the inositol Trisphosphate ($InsP_3$) and the Ryanodine receptors. Most modeling papers on these channels focus on describing their open probability, P_o (i.e., the time the channel is open over the total duration of the experiment), for different ligand concentrations, without paying too much attention to dwell times between openings. However, this latter information is very relevant in order to obtain realistic models of the channels. Mak et al. [1] have studied dwell times in great detail in the case of the $InsP_3$ receptor. In particular, they show in Ref. [1] that the mean open time of the channel ($< T_o >$) is not monotonic with [Ca^{+2}]. On the contrary, it has a concave shape where the maximum corresponds approximately to the [Ca^{+2}] that maximizes the open probability, P_o. In this work, we show that this type of behavior cannot be described using the "classical" description of this ligand-gated channel. We then propose a solution to account for this observation which is based on some features of the receptor's structure.

2. Mean open time

Consider the simplest possible discrete Markov process, x_n, with state space $\{1,0\}$. The transition probabilities for jumping from 1 to 0 is p and from 0 to 1 is q. It is well known that the distribution of 1's in a sequence follows a geometric distribution with parameter p. If the state 1 is associated to the channel's open conformation, then the expected value of the open time (T_o) is $1/p$, independent of what is the value of q or what is the value of the stationary open probability ($\alpha \equiv P_s(1) = q/(p+q)$). What happens if we observe that the mean open time increases monotonically with the open probability? How can we model this? Let us define an independent process, y_n with the same state space and transition probabilities as x_n, and a new process $z_n = \max(x_n, y_n)$. The new process, z_n, is an aggregated Markov process. The state space is aggregated into two observation classes $\{1,0\}$, meanwhile the process $\vec{w_n} \equiv (x_n, y_n)$ is a vectorial Markov chain with state space $\{(1,0),(0,1),(1,1),(0,0)\}$. The observation for $\vec{w_n}(z_n)$ consists of a series of dwell times, $t_1, t_2, \ldots$ during which $\vec{w_n}$ is in a given class (it is in class a during a time, t_1, in class b during t_2, etc.). A fundamental description of such dwell times is the so-called $f_a(k)$, the probability density of a given dwell time k in class a. Colquhoun [2] has derived $f_a(k)$ in the case of two observation classes. For our example, the probability density of the open time $f_1(k)(k = 1,2,\ldots,\infty)$ is given by

$$f_1(k) = \pi_1' Q_{11}^{(k-1)} Q_{10} \,, \tag{1}$$

where π_1' is the vector of equilibrium probabilities defined by

$$\pi_1' = \left(\frac{q(1-q)}{2q-q^2}, \frac{q(1-q)}{2q-q^2}, \frac{q^2}{2q-q^2} \right) \tag{2}$$

and Q_{ij} is the submatrix of the transition probabilities between the states of class i and j (Q_{11} is a 3×3 matrix) of the $\vec{w_n}$ process. The probability of observing the

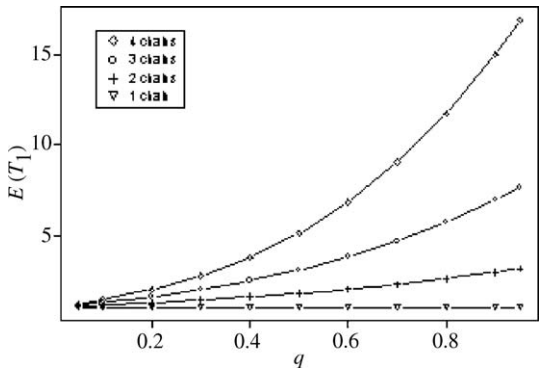

Fig. 1. Expected mean open time ($E(T_1)$) of the maximum process, z_n defined in the text, as a function of q (transition probability from 0 to 1) for $p = 0.9$.

channel open during k time-steps is obtained by multiplying the probability of entering the open state by the probability that it stays open during $(k-1)$ time-steps by the probability of closing. Taking into account Eq. (1) we have found an explicit formula for the expected open time ($E(T_1)$) which is given by

$$E(T_1) = \sum_{k=1}^{\infty} k f_1(k) = \frac{2p+q}{p^2(2-q)} \,. \tag{3}$$

For the case of having d independent processes similar to x_n, we have also found the value of the expected open time ($E(T_1)$). In this case the strategy is different, as we are interested only in the expected open time, and not in the probability density ($f_1(k)$), we mimic the process $z_n = \max(x_n^1, x_n^2, \ldots, x_n^d)$ by a new Markov process $\tilde{z}_n$ with state space $\{1,0\}$, and transition probabilities from 1 to 0 and viceversa, s and t, respectively. This new process $\tilde{z}_n$ has the same expected open time $E(T_1)$ as z_n, but different probability density (geometric). The transition probabilities are given by (see the appendix)

$$r = \frac{\sum_{i=1}^{k} \binom{k}{i} (p\alpha)^i ((1-q)(1-\alpha))^{k-i})}{\sum_{j=1}^{k} \binom{k}{i} (\alpha^i (1-\alpha)^{k-i})} \,, \tag{4}$$

$$s = 1 - (1-q)^k \,. \tag{5}$$

In Eq. (4), α is the stationary open probability ($P_s(open) = P_s(1) = q/(p+q)$) of the x_n process. For $\tilde{z}_n$ process, the distribution of 1's is also geometric but with parameter s. Now, the expected value of the open time for the new process, $1/s$, depends on q since s depends on it. In Fig. 1, we can observe the expected value of the open time, $E(T_1)$, of the maximum process, as a function of q for $p = 0.9$. The different curves correspond to having considered different numbers of independent processes in order to define the maximum process, z_n. The $E(T_1)$ is an increasing function of q, the

transition probability of jumping from 0 to 1. We can also observe that, for a given value of q, the mean open time increases with the number of independent processes. This is easy to understand: when the open probability is very small there is practically no "superposition" of the d independent processes, in the sense that it is very rare that there will be more than one process in the state 1 at any given time. However, when the open probability is greater, there exists superposition between the signals (see the definition of z_n), giving rise to longer runs of 1.

3. $InsP_3$ receptor

In this section, we apply the ideas we have just described to the $InsP_3$ receptor. It is well known that the $InsP_3$ receptor has at least two agonists, Ca^{+2} and $InsP_3$. The first experimental observations in reconstituted lipid bilayers revealed that Ca^{+2} has a dual role [3]: for small concentrations ($\sim$nM) the channel activates, while for greater concentrations ($\sim\mu$M) the channel inactivates. Thus, the open probability has a bell-shape dependence on the calcium concentration. The best-known theoretical model to account for this observation is the De Young–Keizer model [4], where the parameters were determined by fitting the open probability to the experimental data of Ref. [3]. Thus, the model was not based on fitting open or closed times. As mentioned in the Introduction, more recent experiments [1,5,6] have shown that the mean open time has a local maximum as function of $[Ca^{+2}]$.

The $InsP_3$ receptor has four identical subunits. We show in Fig. 2(a), a schematic picture of the De Young–Keizer model for each of these subunits. Each subunit can be in one of eight different states labelled by S_{ijk}, with i, j and $k \in \{0,1\}$). The first index indicates whether $InsP_3$ is bound (1) or not (0) to its site, the second one, whether Ca^{+2} is bound (1) or not (0) to the activatory site and the last one whether Ca^{+2} is bound (1) or not (0) to the inhibitory site. In Ref. [4], the channel is considered to be open if three subunits are in the S_{110} state. It is important to remark that most models of the $InsP_3$ receptor [4,7–9] have the same basic structure regarding the way the channel switches from the open to the closed state (see enlargement in Fig. 2(a)). The channel can close if Ca^{+2} binds to the inhibitory site, or spontaneously, by either losing a Ca^{+2} ion that was bound to an activatory site or by losing $InsP_3$. According to this picture, the mean open time can only be a decreasing function of $[Ca^{+2}]$. However, this contradicts the recent experiments of Refs. [1,5,6]. As we have already mentioned, it is known that, from a structural point of view, the $InsP_3$ receptor is a tetramer [10,11,14] with four identical subunits. Thus, we propose a model in which the four subunits work independently (this is not a necessary condition, in principle, our argument still works even if they are not independent), and assume that the channel opens if at least one subunit is in what we call an open conformation (S_{110}). There is some evidence in favor of the existence of multiple open states. Both in Ref. [12] and in Refs. [6,13] various subconductance levels are shown, which is an indication that the channel can be open in more than one conformation. As we show now, by assuming that the channel may have more than one open conformation we can easily explain the non-monotone dependence of the mean open time with the calcium concentration. In

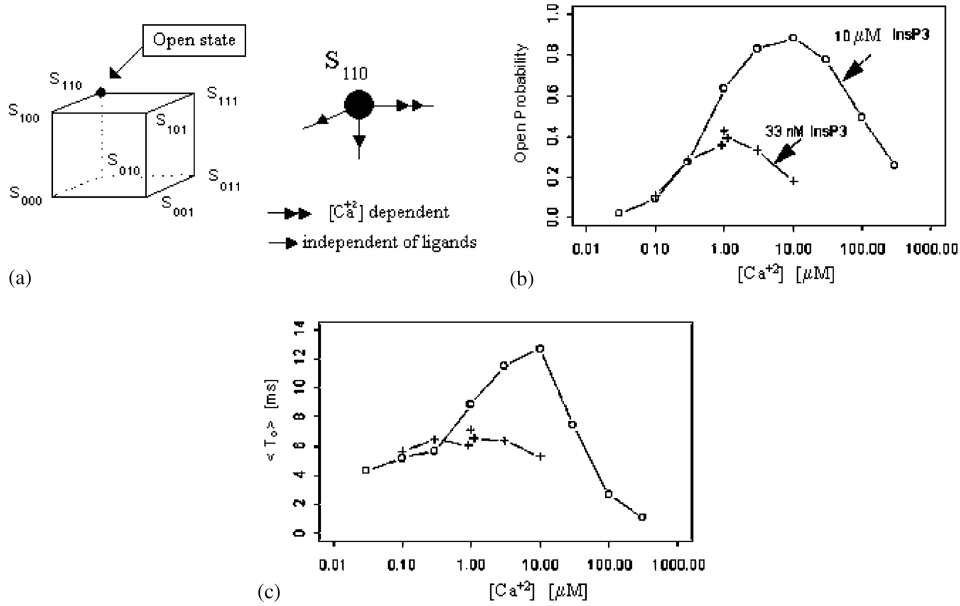

Fig. 2. (a) De Young–Keizer model for each subunit of the $InsP_3$ receptor. (b) Open probability as a function of $[Ca^{+2}]$ for two values of $[InsP_3] = 10$ μM, and 33 nM. (c) Mean open time (ms) of the channel assuming that the subunits work independently.

order to show that our approach can be the solution to help explain the observations of Refs. [1,5,6] we have used the De Young–Keizer model for each of the receptor's subunits. To this end, we simulate the process $w_n = \max(x_n^1, x_n^2, x_n^3, x_n^4)$, where x_n^i are independent Markov chains with state space aggregated into two classes $\{0, 1\}$. Each chain is generated using the De Young–Keizer model (see Fig. 2(a)) and associating the state 1 (open) each time the subunit is in the S_{110} state and 0 (closed) otherwise. We show in Fig. 2(b), the open probability we obtain from these simulations for two values of $[InsP_3]$, and in Fig. 2(c) the mean open time as a function of $[Ca^{+2}]$. The open probability is similar to the experimentally observed one depicted in Ref. [1]: the shape and range of values are very similar. We can observe in Fig. 2(c) that the mean open time is also very similar to the experimental one of Ref. [1], and that it has a local maximum as a function of $[Ca^{+2}]$ that occurs at the calcium concentration that maximizes the open probability. For $[InsP_3] = 10$ μM we can observe that, for small $[Ca^{+2}]$, the mean open time has a constant value of $\sim$4 ms. This is the value of the mean open time of a single subunit (x_n^1). As the $[Ca^{+2}]$ is increased, the mean open time also increases, because of the superposition of the signals. However, it then decreases because the open probability of each subunit decreases, going to zero because of the inhibitory effect of large $[Ca^{+2}]$. On the other hand, for $[InsP_3] = 33$ nM the effects $[Ca^{+2}]$ on the mean open time, $\langle T_o \rangle$, are small because the open probability is small.

4. Conclusions

We have addressed the issue of how the mean open time of an ion channel can depend non-monotonically on the concentration of one of its ligands. In particular, we have analyzed the case of the $InsP_3$ receptor, which is a ligand-gated calcium channel that is involved in several physiological processes. Recent experiments have shown that the mean open time of this channel has a local maximum as a function of $[Ca^{+2}]$. This behavior cannot be accounted for by the usual models of the receptor that have been published in the literature. We have shown that we can explain this behavior if we consider the tetrameric structure of the channel and assume that it can have more than one open conformation (when any of the subunits is in an open state). We have addressed the problem within a general mathematical framework in terms of Markov chains and we have also presented numerical simulations for the case of the $InsP_3$ receptor.

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Appendix

For d independent processes we have that the transition probability from 1 to 0, $r = P(z_{n+1} = 0/z_n = 1) = P(z_{n+1} = 0 \cap z_n = 1)/P(z_n = 1)$. Now if $z_n = 1$, then there is at least one chain that is in the 1 state. So we can split this up into disjoint events $A_1, A_2, A_3, \ldots, A_d$ where the event A_i is defined as the event in which there are only i chains in state 1. Now using that the chains are independent and the fact that the process x_n has a unique stationary solution we obtain Eq. (4).

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