Contribution of Persistent Na$^+$ Current and M-Type K$^+$ Current to Somatic Bursting in CA1 Pyramidal Cells: Combined Experimental and Modeling Study

David Golomb, Cuiyong Yue, and Yoel Yaari

Department of Physiology and Zlatowski Center for Neuroscience, Faculty of Health Sciences, Ben-Gurion University, Be’er-Sheva, Israel; and Department of Physiology, Institute of Medical Sciences, Hebrew University-Hadassah Faculty of Medicine, Jerusalem, Israel

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Golomb, David, Cuiyong Yue, and Yoel Yaari. Contribution of persistent Na$^+$ current and M-type K$^+$ current to somatic bursting in CA1 pyramidal cells: combined experimental and modeling study. J Neurophysiol 96: 1912–1926, 2006. First published June 28, 2006; doi:10.1152/jn.00205.2006. The intrinsic firing modes of adult CA1 pyramidal cells vary along a continuum of “burstiness” from regular firing to rhythmic bursting, depending on the ionic composition of the extracellular milieu. Burstiness is low in neurons exposed to a normal extracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]o), but is markedly enhanced by lowering [Ca$^{2+}$]o, although not by blocking Ca$^{2+}$- and Ca$^{2+}$-activated K$^+$ currents. We show, using intracellular recordings, that burstiness in low [Ca$^{2+}$], persists even after truncating the apical dendrites, suggesting that bursts are generated by an interplay of membrane currents at or near the soma. To study the mechanisms of bursting, we have constructed a conductance-based, one-compartment neuron model of CA1 pyramidal neurons. In this neuron model, reduced burstiness in low [Ca$^{2+}$], is simulated by negatively shifting the activation curve of the persistent Na$^+$ current (I_{NaP}) as indicated by recent experimental results. The neuron model accounts, with different parameter sets, for the diversity of firing patterns observed experimentally in both zero and normal [Ca$^{2+}$]o. Increasing I_{NaP} in the neuron model induces bursting and increases the number of spikes within a burst but is neither necessary nor sufficient for bursting. We show, using fast-slow analysis and bifurcation theory, that the M-type K$^+$ current (I_{M}) allows bursting by shifting neuronal behavior between a silent and a tonically active state provided the kinetics of the spike generating currents are sufficiently, although not extremely, fast. We suggest that bursting in CA1 pyramidal cells can be explained by a single compartment “square bursting” mechanism with one slow variable, the activation of I_{Na}.  

INTRODUCTION

The intrinsic discharge mode of individual cortical pyramidal cells varies along a spectrum of “burstiness,” from regular firing evoked by depolarization of the neuron to spontaneous bursting unprovoked by any extrinsic stimuli (Jensen et al. 1994; Schwartzkroin 1975; for a detailed description of firing and bursting patterns, see Fig. 1 in Su et al. 2001). A large body of evidence now indicates that the propensity of a neuron to burst depends not only on its constitution, i.e., the nature and properties of ionic conductances expressed in its plasma membrane, but also on its environment, i.e., the ionic composition of the milieu in which it is embedded. Thus regular firing pyramidal cells readily convert to a bursting mode when the extracellular concentrations of Ca$^{2+}$ ([Ca$^{2+}$]o) or K$^+$ ([K$^+$]o) decrease or increase, respectively (Jensen et al. 1994; Su et al. 2001). Such changes in extracellular ion composition accompany neuronal activity (Heinemann et al. 1977), which may explain why the propensity for bursting of pyramidal cells increases with the level of activity in their surrounds (Harris et al. 2001). Interestingly, blocking Ca$^{2+}$ currents or Ca$^{2+}$-activated K$^+$ currents (Azouz et al. 1996; Jensen et al. 1994; Su et al. 2001) does not increase the propensity for bursting. Given that the bursting mode plays important roles in electrical signaling, normal and abnormal neuronal synchronization, and induction of long-term synaptic plasticity (Izhikevich et al. 2003; Lisman 1997; Yaari and Beck 2002), it is important to understand how constitution and environment interact in regulating this discharge mode. To date, most theoretical studies of intrinsic bursting have focused on the former factor.

In neocortical, subicular, and CA3 pyramidal cells, intrinsic bursting has been attributed to recruitment of Ca$^{2+}$ currents by the primary Na$^+$ spike (Chen et al. 2005; Junger et al. 2001; Larkum et al. 1999; Metz et al. 2005; Wong and Prince 1981). In line with these experimental observations, many models of Ca$^{2+}$ current-dependent bursting suggest a “ping-pong” interplay between fast Na$^+$ and K$^+$ currents in the soma and slow Ca$^{2+}$ and K$^+$ (mostly Ca$^{2+}$-dependent) currents in the apical dendrites (Mainen and Sejnowski 1996; Pinsky and Rinzel 1994; Traub and Miles 1991; Traub et al. 1991, 1994; Warman et al. 1994). In adult CA1 pyramidal cells, however, bursting behavior persists after almost complete truncation of the apical dendrites (Yue et al. 2005). Therefore the mechanism of bursting in the latter neurons may be different from the ping-pong mechanism, which depends on the integrity of apical dendrites.

We study the mechanism by which changes in [Ca$^{2+}$]o modulate transitions between regular firing and bursting in adult CA1 pyramidal cell by combining electrophysiological, computational, and analysis techniques.

METHODS

Hippocampal slices

All animal experiments were conducted in accordance with the guidelines of the Animal Care Committees of the Hebrew University. Adult male Sabra rats were decapitated under deep isoflurane anes-
the applied current. The ionic currents are: \( I_{\text{Na}}(V, h) = g_{\text{Na}}m^3(V)(V - V_{\text{Na}}), I_{\text{NaP}}(V) = g_{\text{NaP}}P_d(V)(V - V_{\text{Na}}), I_{\text{K}}(V, n) = g_{\text{K}}n^4(V - V_{\text{K}}), I_\text{sAHP}(V, b) = g_{\text{sAHP}}(V - V_{\text{K}}), I_{\text{Q}}(V, z) = g_{\text{Q}}(V - V_{\text{Q}})) \). The conductances and reversal potentials are: \( g_{\text{Na}} = 35 \text{ mS/cm}^2, g_{\text{K}} \) varies between 0 and 0.41 \text{ mS/cm}^2, \( g_{\text{NaP}} = 6 \text{ mS/cm}^2, g_{\text{Q}} = 1.4 \text{ mS/cm}^2, g_{\text{M}} = 1 \text{ mS/cm}^2, V_{\text{Na}} = 55 \text{ mV}, V_{\text{K}} = -90 \text{ mV} \). The kinetics parameters and lists are presented in Table 1. The cell model for zero \([\text{Ca}^{2+}]_i\) has five dynamical variables: \( V, h, b, z \), and \( \zeta \).

MODEL FOR NONZERO \([\text{Ca}^{2+}]_i\). This second model includes all the currents that belong to the first model, with the same parameters (except for the effect of \([\text{Ca}^{2+}]_i\) on \( h \)), the half-maximum potential of \( I_{\text{NaP}} \); see Table 1). In addition, we added three more ionic currents: the highest-threshold \( \text{Ca}^{2+} \) current \( I_{\text{Ca}} \), and two \( \text{Ca}^{2+} \)-activated \( \text{K} \) currents, namely, the fast \( \text{Ca}^{2+} \)-activated \( \text{K} \) current \( (I_{\text{KCa}}) \) which contributes to rapid spike repolarization (Storm 1987), and the slow \( \text{Ca}^{2+} \)-activated \( \text{K} \) current \( (I_{\text{Ahp}}) \), which mediates a slow afterhyperpolarization (AHP) and spike frequency adaptation (Madison and Nicoll 1984). This model includes, therefore, the most important currents known in soma and proximal axons of CA1 cells. The current balance equation in this variation of the model is

\[
\frac{dV}{dt} = -g_L(V - V_L) - I_{\text{Na}} - I_{\text{K}} - I_{\text{Ca}} - I_{\text{Ahp}} - I_{\text{APP}} (2)
\]

The ionic currents are: \( I_{\text{Ca}}(V, r) = g_{\text{Ca}}r^2(V - V_Ca), I_{\text{Ca}}(V, c) = g_{\text{Ca}}d_c((\text{Ca}^{2+})^c)(V - V_Ca), I_{\text{Ahp}}(V, q) = g_{\text{Ahp}}q(V - V_Ca). \) The conductances and reversal potentials are: \( g_{\text{Ca}} \) is typically between 0 and 0.2 \text{ mS/cm}^2, \( g_{\text{M}} = 10 \text{ mS/cm}^2, g_{\text{Ahp}} = 5 \text{ mS/cm}^2, V_{\text{Ca}} = 120 \text{ mV} \). The kinetics equations and parameters are listed in Table 2. The dynamics of the calcium concentration inside the cell, \([\text{Ca}^{2+}]_i\), are

\[
\frac{d[\text{Ca}^{2+}]}{dt} = -u_{\text{Ca}}[\text{Ca}^{2+}]/\tau_{\text{Ca}} (3)
\]

\( v = 0.13 \text{ cm}^2/(\text{ms} \times \mu\text{A}), \tau_{\text{Ca}} = 13 \text{ ms} \). The variable \([\text{Ca}^{2+}]_i\) in our model is dimensionless (Traub et al. 1994). It is proportional to the \( \text{Ca}^{2+} \) concentration in a thin internal cylindrical shell adjacent to the membrane.

The low-threshold T-type \( \text{Ca}^{2+} \) current was not included in the model because it is localized mostly in the distal apical dendrites of adult CA1 pyramidal cells (Karst et al. 1993; Thompson and Wong 1991). The hyperpolarization-activated cationic current \( (I_{\text{h}}) \) (Magee 1998; Vasiliev and Barish 2002) was not included in the model because its density in the soma is much lower than in the apical dendrites (Magee 1998) and because the activation kinetics of the largest component of \( I_{\text{h}} \) are faster than that of \( I_{\text{M}} \) (Maccabelli and McBain 1996; Magee 1998; Spain et al. 1987) and cannot support bursting via a mechanism based on two slow variables (Bertram et al. 1995). The apamin-sensitive, small-conductance (SK) \( \text{Ca}^{2+} \)-activated \( \text{K} \) current \( (I_{\text{Ahp}}) \), which can be evoked under voltage-clamp conditions in CA1 pyramidal cells (Gu et al. 2005; Stocker et al. 1999), was not included in the model. The reason for this exclusion is that in current-clamp recordings this current does not contribute appreciably to spike frequency adaptation and the medium AHP (Gu et al. 2005).

NUMERICAL METHODS. Simulations were performed using the fourth-order Runge-Kutta method with a time step of 0.05 ms implemented as a C program or within the software package XPPAUT (Ermentrout 2002), that was used also for computing bifurcation diagrams.

STIMULATION. We analyzed the firing patterns of the neuron model in response to two types of stimuli, namely, brief and prolonged square positive current pulses. In the former case, pulse duration was 3 ms, so it could evoke a spike response without interfering with spike afterpotentials (Su et al. 2001; Yue and Yaari 2004). In the latter case,
The activation and inactivation curves \( x_i(V) \) are determined by the equation \( x_i(V) = \frac{1}{1 + \exp[-(V - \theta_i)/\alpha_i]} \) for \( i = m, h, n, a, b, z \). References and comments: 1) Fleidervish et al. (1996), Martina and Jonas (1997); Sah et al. (1998b); and Golomb and Amitai (1997). The difference between \( \theta_i \) and \( \alpha_i \) is smaller than what is usually measured in pyramidal cell somata, partially because the activation curve of axons in pyramidal cells is shifted toward more hyperpolarized values (Colbert and Pan 2002). 2) French et al. (1990). In the cerebellum (Kay et al. 1998) and the entorhinal cortex (Magistretti et al. 2003), these kinetics were found to be as fast as the kinetics of \( I_{Na} \) in the submillisecond regime time scale. Therefore we consider the kinetics of \( I_{Ca} \) to be instantaneous. Recent experiments in CA1 pyramidal cells show that reducing \( \text{Ca}^{2+} \) concentration leads to a significant negative shift to \( \theta_i \) (Yue et al. 2005; see also Li and Hatton 1996). Therefore, \( \theta_i \) is varied throughout the paper (typically between \(-47 \text{ mV at } 0 \text{ [Ca}^{2+} \text{]} \), and \(-41 \text{ mV at physiological [Ca}^{2+} \text{]} \), values). 3) Martina et al. (1998); Sah et al. (1998a); 4) Hoffman et al. (1997); Rush and Rinzel (1995); and 5) Halliwell and Adams (1982).

### Fast-slow analysis

We used the fast-slow method (Bertram et al. 1995; Hoppensteadt and Izhikevich 1997; Izhikevich 2000, 2006; Rinzel and Ermentrout 1998) to study the bursting mechanism, to determine the role of the currents \( I_{Na} \) and \( I_{M} \) in the dynamics, and to determine the necessary conditions for bursting. This method has been applied successfully to analyze periodic bursting of various biophysical models of neurons (e.g., Mandelblat et al. 2001). We applied the method for the case \( \text{[Ca}^{2+}] \_o = 0 \) (and thus \( \theta_p = -47 \text{ mV and } \text{Ca}^{2+}_o = 0 \) because it has simpler dynamics. The analysis is based on separating the dynamical variables of the system into two subsystems, “fast” and “slow.” The model has five dynamic variables: \( V, h, n, a, z \). The first four variables are considered to be “fast” and belong to the fast subsystem. The variable \( z \) is considered to be “slow.” The analysis is exact in the limit \( z \rightarrow \infty \), where \( z \) is the time constant of \( z \). It describes an approximation of the dynamics for large but finite \( z \). In the first stage of the analysis, the bifurcation diagram of the fast subsystem was computed with the slow variable \( z \) considered as a parameter. In the second stage, the dynamics of \( z \) itself were computed using the time-averaged values of the fast subsystem.

If the dynamics of the fast subsystem converges to a stable rest state (fixed point), \( z \) is determined by its value for the fixed point of the fast subsystem for the instantaneous value of \( z \), denoted by \( z_{\text{rest,fast}}(\zeta) \), whereas \( z \) evolves slowly (Table 1). If \( z_{\text{rest,fast}}(\zeta) > z \), \( z \) increases.
slowly, and if \( z_{\text{equiv}}(z_{\text{LC}}) < z \), \( z \) decreases slowly. If, for a certain value of \( z \) denoted by \( z_{\text{FP}} \), \( z_{\text{equiv}}(z_{\text{FP}}) = z_{\text{FP}} \), namely, the line representing the stable rest state, \( V_{\text{equiv}}(z) \), intersects with \( z_{\text{equiv}}(V) \), the activation curve of \( z \) (Table 1), the point \( [V_{\text{equiv}}(z_{\text{FP}}), z_{\text{FP}}] \) represents a fixed point of the full system. It is stable if \( dV_{\text{equiv}}(z)/dz < [dz_{\text{equiv}}(V)/dV]^{-1} \). The dynamics of the fast subsystem may converge also to a stable tonic firing state (limit cycle), where \( T_r \) is the time period of the cycle that depends on \( z \). The approximation that \( \tau_r \) is much larger than the other time scales in the system, and, specifically, \( \tau_r > T_r \), means that \( z \) does not vary significantly during one cycle of the fast subsystem. Therefore we can average the dynamical equation for \( dz/dt \) (Table 1) over one cycle of the fast, tonic firing to obtain (Bertram et al. 1995; Izhikevich 2006; Mandelblat et al. 2001)

\[
\langle dz/dt \rangle = \langle [z_{\text{equiv}}(V) - z]/\tau_r \rangle \tag{4}
\]

where \( \langle \ldots \rangle \) denotes time average over a period \( T_r \) and \( V_{\text{equiv}} \) is defined implicitly by the equation

\[
z_{\text{equiv}}(V) = \frac{1}{T_r} \int_0^{T_r} z_{\text{equiv}}(V(t)) \, dt \tag{5}
\]

In the slow time scale, we can replace \( dz/dt \) by \( dz/dt \). Therefore the dynamics of the slow variable \( z \) is determined, on average, by the right hand side of Eq. 5. If \( z_{\text{equiv}}(z_{\text{LC}}) > z \), \( z \) increases slowly, and if \( z_{\text{equiv}}(z_{\text{LC}}) < z \), \( z \) decreases slowly. We consider the case where, for a certain value of \( z \) denoted by \( z_{\text{LC}} \), the line representing \( V_{\text{equiv}}(z) \) intersects with the activation curve of \( z \), namely \( z_{\text{equiv}}(V_{\text{equiv}}(z_{\text{LC}})) = z_{\text{LC}} \). In this case, the point \( (V_{\text{equiv}}(z_{\text{LC}}), z_{\text{LC}}) \) represent a limit cycle of the full system. It is stable if \( dV_{\text{equiv}}(z)/dz < [dz_{\text{equiv}}(V)/dV]^{-1} \). In particular, the limit cycle is stable if the slope of \( V_{\text{equiv}}(z) \) at \( z = z_{\text{LC}} \) is negative.

**RESULTS**

**Effects of lowering \([Ca^{2+}]_o\) on truncated CA1 pyramidal cells**

We have recorded from 24 CA1 pyramidal cells after truncation of their apical dendrites. When activated with brief (3–4 ms) depolarizing current pulses, truncated neurons displayed normal spikes followed by a distinct, albeit variable in size, afterdepolarization (ADP; Fig. 1A, left). The mean (±SE) resting potential, apparent input resistance, and spike amplitude of these neurons were \(-67.8 ± 0.5 \text{ mV} \) (range, \(-75 \text{ to } -61 \text{ mV} \)), \( 40.1 ± 2.0 \text{ MΩ} \) (range, \( 20.5 \text{ to } 48.7 \text{ MΩ} \)), and \( 92.1 ± 5.7 \text{ mV} \) (range, \( 71.7 \text{ to } 103.2 \text{ mV} \)), respectively. These values are well within the values obtained for intact neurons (Yue and Yaari 2004; Yue et al. 2005) and indicate that despite the damage inflicted by the cut and loss of most apical dendrites, the electrophysiological properties of the axo-soma are well preserved. When activated with prolonged (200 ms) depolarizing current pulses of increasing intensities, the neurons discharged repetitively and displayed spike frequency adaptation (Fig. 1B, left). Like in intact neurons (Su et al. 2001; Yue et al. 2005), approximately half of the neurons were
neurons, we tested the effects of the bursting (Fig. 2) and converted rhythmic bursting during spikes (Fig. 3). Depolarizing pulses, the neuron generated a burst of three spikes (Fig. 3) and 5

Effects of blocking persistent Na current

A previous study in intact CA1 pyramidal cells concluded that the augmented spike ADPs and bursting behavior in low [Ca\(^{2+}\)]\(_{o}\) are driven predominantly by persistent Na current (I_{NaP}) (Su et al. 2001). To extrapolate this finding to truncated neurons, we tested the effects of the I_{NaP} blocker riluzole (Yue et al. 2005) on bursting induced by low [Ca\(^{2+}\)]\(_{o}\) in five such neurons. Riluzole (10 µM) blocks I_{NaP} in CA1 pyramidal cells almost completely while exerting a lesser effect on the transient Na current (Spadoni et al. 2002; Urban et al. 2000; Yue et al. 2005). Representative results are shown in Fig. 2. Adding 10 µM riluzole to the ACSF abolished low Ca\(^{2+}\)-induced bursting (Fig. 2A) and converted rhythmic bursting during prolonged depolarizations to regular firing (Fig. 2B). Similar results were obtained (data not shown) with 100 µM phenytoin (n = 3) and 5 µM PDB (n = 2), which also block I_{NaP} (Cantrill et al. 1996; Chao and Alzheimer 1995; Yue et al. 2005). Together, these data strongly suggest that bursting in low [Ca\(^{2+}\)]\(_{o}\) is driven predominantly by I_{NaP}.

Effects of blocking M-type K current

Because inactivation of I_{NaP} in CA1 pyramidal cells is very slow (in the order of seconds) (French et al. 1990), burst termination likely involves activation of an outward K current. In nominally Ca\(^{2+}\)-free ACSF, Ca\(^{2+}\)-activated K currents are inoperative. Hence a likely candidate for burst termination in this condition is the M-type K current (I_{M}), previously shown to activate during the spike ADP and repolarize the neuron back to its resting potential (Yue and Yaari 2004). We tested this notion using linopirdine and XE991, selective blockers of I_{M} (Schnee and Brown 1998; Wang et al. 1998). Representative results from one truncated neuron bathed in Ca\(^{2+}\)-free ACSF are shown in Fig. 3. When activated by brief depolarizing pulses, the neuron generated a burst of three spikes (Fig. 3A, left). Adding 10 µM linopirdine to the Ca\(^{2+}\)-free ACSF markedly prolonged the burst and delayed repolarization of the neuron for several hundreds of milliseconds (Fig. 3A, middle). Both the primary burst and the underlying plateau potentials were readily suppressed by subsequent addition of 10 µM riluzole to the ACSF (Fig. 3A, right), suggesting that they are driven by I_{NaP}. Similar results were obtained when the neuron was stimulated with prolonged depolarizing current pulses that induced rhythmic bursting (Fig. 3B, left). Under the influence of linopirdine, rhythmic bursting converted to a plateau depolarization that lasted throughout the period of stimulation (Fig. 3B, middle). Again, adding 10 µM riluzole to the ACSF suppressed the initial burst responses and the plateau depolarizations and imposed a regular firing mode (Fig. 3B, right). Similar results were obtained in five truncated neurons treated with 10 µM linopirdine and two truncated neurons treated with 3 µM XE991. Together these data suggest that bursting in truncated neurons bathed in Ca\(^{2+}\)-free ACSF is due to interplay between I_{NaP} and I_{M}.

Construction of an experimentally based model

We have constructed a model of CA1 pyramidal cells to further understand the mechanisms underlying their somatic bursting. The results of the model are compared with the experimental results from the following aspects. First, can the model account for the variant firing patterns observed experimentally as its parameters are varied? Second, do the propen-
sity for bursting and $N_S$ vary in a similar manner in the model and in the experiments as $I_{app}$, $g_{NaP}$, or $g_M$ vary? Finally, are the influences of $g_{Ca}$, $g_C$, and $[Ca^{2+}]_o$ on the propensity for bursting and $N_S$ similar in the model and in the experiments? We have included in the model neuronal dynamics with only two time scales, namely that of spikes (a few milliseconds) and that of bursting (a few hundreds of milliseconds). Experimentally, we found that many CA1 pyramidal cells also exhibited very slow dynamics (a few seconds) that may cause rhythmic bursting to change eventually to repetitive spikes (Figs. 1B, middle, and 3B, left, 2 traces). The very slow dynamics, however, often reached a steady state. In those cases, the neurons displayed rhythmic bursting continuously, as shown in Fig. 4A. Therefore we omitted the very slow dynamics from the model, allowing it also to manifest rhythmic bursting (Fig. 4B).

**Fast-slow analysis of the necessary conditions for bursting and the roles of $I_{NaP}$ and $I_M$**

We have shown in the preceding text that two currents, namely $I_{NaP}$ and $I_M$, play essential roles in bursting in truncated CA1 pyramidal cells bathed in 0 $[Ca^{2+}]_o$. The $I_{NaP}$ is considered here to be nonactivating (at the relevant time scales), and its activation variable, $p$, is considered to be instantaneous and equal to $p_\infty(V)$. The activation variable of $I_M$, $z$, is relatively slow, with time constant $\tau_z = 75$ ms. Therefore we used the fast-slow method in the condition that $z$ is the only slow variable.

To illuminate the contribution of $I_{NaP}$ to bursting, we carried out the analysis for four values of $g_{NaP}$: 0 (Fig. 5A), 0.2 mS/cm$^2$ (Fig. 5B), 0.3 mS/cm$^2$ (Fig. 5C), and 0.41 mS/cm$^2$ (Fig. 5D). In all panels, the bifurcation diagrams of the fast subsystem are computed with $z$ considered as a parameter. The steady state (fixed point; thin black line) is stable for large $z$. This stable rest state coalesces with an unstable state and ceases to exist in a saddle-node bifurcation. The interval of time between the spikes of the oscillatory state (limit cycle) is a function of $z$. The oscillatory state (limit cycle) emerges, corresponding to tonic, periodic firing. This oscillation occurs...
The bursting mechanism in our model depends on the bistability of the fast subsystem. To obtain bistability, there should be strong-enough inward current that is active in steady state at membrane potentials around spike threshold, namely $[g_{NaP}m^{\infty}_v(V)h_v(V) + g_{NaP}p_v(V)](V - V_{Na})$ should be large enough for $V$ around spike threshold. In our model, this inward current can be generated by $I_{NaP}$ or the window $I_{Na}$. In addition, the minimal voltage of the fast subsystem during the tonic state should be depolarized enough (above the thin dotted black line representing the unstable rest state). Therefore the kinetics of the currents underlying spike depolarization and repolarization ($I_{Na}$ and $I_{Kdr}$ in our model) should be fast enough (Bertram et al. 1995). Indeed, a previous model in which $h$ and $n$ were 3.7 times slower than in the present model did not display bursting behavior (Golomb and Amitai 1997). If the kinetics of these variables are too fast, however, the fast subsystem will exhibit a high plateau depolarization. As a result, the neuronal voltage will oscillate between a rest state and a high plateau depolarization instead of exhibiting bursts of spikes.

2. STRENGTH OF $g_{NaP}$ The effect of $I_{NaP}$ on the tonic state is larger than its effect on the rest state because this current is activated at depolarized membrane potentials (Table 1). As $g_{NaP}$ increases, the tonic state is shifted more to the right.

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**FIG. 4.** Comparison of spontaneous rhythmic bursting in a truncated CA1 pyramidal cell and in the neuron model. A: experiment. In this truncated neuron, perfusing the slice with nominally Ca$^{2+}$-free ACSF induced spontaneous rhythmic bursting. B: modeling. An example of spontaneous bursting ($I_{app} = 0 \mu A/cm^2$) in the neuron model at $[Ca^{2+}]_o = 0$. Other parameters are: $g_{NaP} = 0.3 \text{ mS/cm}^2; V_L = -62 \text{ mV}$. We then analyzed the behavior of the full system of equations defining the neuronal dynamics. For $g_{NaP} = 0$ (Fig. 5A), the oscillatory state is the only stable state for small (positive) $z$ values, and the rest state is the only stable state for larger $z$ values. There is a tiny regime of bistability where both states are stable, but for our description, it can be ignored. For $g_{NaP} = 0.2 \text{ mS/cm}^2$ (B), the bistable regime, in which both the rest state and the oscillatory state exist and are stable, is more extended. For $g_{NaP} = 0.3 \text{ mS/cm}^2$ (C), the oscillatory solution extends toward the right, coalesces with an unstable periodic state, and this unstable state coalesces with another stable oscillatory state with a larger amplitude. For $g_{NaP} = 0.41 \text{ mS/cm}^2$ (D), the Hopf bifurcation point is shifted to the right, and there is bistability between a rest state and a depolarized plateau. The stable oscillatory state with large amplitude almost disappears, and the amplitude of the remaining oscillatory state is small.

We then analyzed the behavior of the full system of equations defining the neuronal dynamics. For $g_{NaP} = 0$ (Fig. 5A), the bistable regime is tiny and cannot generate bursting states with the present kinetics of $z$; the neuron fires tonically. For $g_{NaP} = 0.2 \text{ mS/cm}^2$ (Fig. 5B), there is a significant bistable regime. When the neuron fires, $z$ slowly increases until it reaches beyond the bistable regime. The neuron dynamics goes to the rest state, where $z$ decreases. When $z$ reaches the “knee” where the stable rest state disappears, the dynamics goes back to the firing state and a new bursting cycle begins. Similar behavior occurs for $g_{NaP} = 0.3 \text{ mS/cm}^2$ (Fig. 5C) except that the number of spikes ($N_a$) increases because of the more extended bistable regime. The system does not settle into the fast oscillations with small amplitude for this parameter value. For $g_{NaP} = 0.41 \text{ mS/cm}^2$ (Fig. 5D), the system first settles into the high plateau, and the amplitude of the oscillations decreases with time. As $z$ increases, the state switches to the fast oscillations with small amplitude, and the amplitude of the oscillations increases with time until the system switches to the rest state. This scenario leads to bursting state with fast, low-amplitude spikes whose amplitude first decreases and then increases with time (see following text, Fig. 8B, III).

From the fast-slow analysis, we derive several conclusions regarding the neuronal firing dynamics.

1. BISTABILITY OF THE FAST SUBSYSTEM. The bursting mechanism in our model depends on the bistability of the fast subsystem. To obtain bistability, there should be strong-enough inward current that is active in steady state at membrane potentials around spike threshold, namely $[g_{NaP}m^{\infty}_v(V)h_v(V) + g_{NaP}p_v(V)](V - V_{Na})$ should be large enough for $V$ around spike threshold. In our model, this inward current can be generated by $I_{NaP}$ or the window $I_{Na}$. In addition, the minimal voltage of the fast subsystem during the tonic state should be depolarized enough (above the thin dotted black line representing the unstable rest state). Therefore the kinetics of the currents underlying spike depolarization and repolarization ($I_{Na}$ and $I_{Kdr}$ in our model) should be fast enough (Bertram et al. 1995). Indeed, a previous model in which $h$ and $n$ were 3.7 times slower than in the present model did not display bursting behavior (Golomb and Amitai 1997). If the kinetics of these variables are too fast, however, the fast subsystem will exhibit a high plateau depolarization. As a result, the neuronal voltage will oscillate between a rest state and a high plateau depolarization instead of exhibiting bursts of spikes.
Effects of varying $g_{\text{NaP}}$

CA1 pyramidal cells perfused with Ca$^{2+}$-free ACSF display a diversity of firing patterns, ranging from regular firing to spontaneous rhythmic bursting (Figs. 1–3 and 4A) (Azouz et al. 1996; Su et al. 2001). We first tested the hypothesis that a variation in the density of persistent Na$^+$ channels may generate such diversity. We assumed that in this condition $\theta_p = -47\text{ mV}$ and examined the consequences of increasing $g_{\text{NaP}}$. When $g_{\text{NaP}}$ was set to zero, the neuron fired in a regular mode during sustained depolarization (Fig. 6A). As $g_{\text{NaP}}$ was raised, the propensity for bursting increased, so that at $g_{\text{NaP}} = 0.08 \text{ mS/cm}^2$, the neuron became a periodic burster when strongly depolarized (Fig. 6B) and at $g_{\text{NaP}} = 0.18 \text{ mS/cm}^2$, it burst-fired periodically in response to all suprathreshold stimuli (Fig. 6C). Further increasing $g_{\text{NaP}}$ to 0.3 mS/cm$^2$ increased the burstiness of the neuron, so it now fired a burst also in response to brief stimuli (Fig. 6D). Changing $V_1$, from −70 to −62 mV destabilized the rest state and caused spontaneous rhythmic bursting when $g_{\text{NaP}}$ was high (Fig. 4B). For $g_{\text{NaP}} = 0$, the neuron was quiescent even for $V_1 = -62\text{ mV}$.

In all the cases shown in Figs. 4B and 6, the number of spikes in the first burst was larger than or equal to $N_S$, the average number of spikes in later bursts. The reason for that is that $z$, the activation variable of $I_M$, is nearly zero at the beginning of the stimulus but increases later, so the limiting effect of $I_M$ on the number of spikes is expressed with a delay.

The analysis is carried out in the limit that $\tau_z$ is much larger than all the other time constants, even though in reality it is not extremely large ($\tau_z = 75\text{ ms}$). Therefore it is necessary to examine whether the conditions for bursting obtained using the analysis are still valid for this value of $\tau_z$. In the following subsections, we turn back to the full system and study it using numerical simulations. We also examine whether the model can account for the variant firing patterns of CA1 pyramidal cells.
To further characterize the neuron model behavior, we have drawn a map of $N_s$ in bursts evoked by brief (3 ms; Fig. 7A) and prolonged (Fig. 7B) stimuli in the parameter plane of $g_{NaP}$ and $I_{app}$. All other parameters were fixed as in the reference parameter set. Brief pulses evoked only a single spike if $g_{NaP}$ was zero or small. As $g_{NaP}$ increased, $N_s$ increased as predicted by the fast-slow analysis. For very large $g_{NaP}$, the neuron fired many fast and short-amplitude spikes, almost converging to a high plateau, before going back to rest (Fig. 7B, top right). The borders of the regimes of 4, 5, 6, etc. spikes were almost independent of $I_{app}$ when it was not too large. This is consistent with the common experimental observation that when $I_{app}$ is brief, $N_s$ is independent of $I_{app}$. The map for bursts evoked by prolonged current pulses also showed that $N_s$ increases with $g_{NaP}$ (Fig. 7B). The parameter $I_{app}$ had a larger impact on $N_s$ in comparison with the case of brief pulses, so that increasing it could raise $N_s$ by one or two spikes.

We denoted \( f \) to be the frequency of periodic bursts evoked by prolonged depolarizations. For a given $N_s$, \( f \) increased as $g_{NaP}$ or $I_{app}$ increased (Fig. 7, C and D) because the neuron was more excitable. At $g_{NaP}$ or $I_{app}$ values at which $N_s$ increased by 1, \( f \) decreased abruptly because more prolonged bursts were followed by larger AHPs.

COMPARING EXPERIMENTAL RESULTS, MODELING, AND THEORY. Increasing $g_{NaP}$ increases $N_s$ and eventually generates bursts with fast, low-amplitude spiking both in the analysis (Fig. 5) and the simulations (Fig. 7). Similarly, blocking $g_{NaP}$ decreases $N_s$ and eventually eliminates bursting in response to prolonged current pulses in the experiments (Fig. 2), the simulations (Figs. 6 and 7) and the mathematical analysis (Fig. 5). The same dependency $N_s$ on $g_{NaP}$ is found experimentally and computationally in response to brief pulses. Furthermore, in response to prolonged pulses, the number of spikes in the first burst is larger than the number of spikes in subsequent bursts in both experiments and simulations.

Role of $g_M$

Our data (Fig. 3), together with previous data about intact neurons (Yue and Yaari 2004, 2006), show that selective block of $I_M$ markedly enhances the burstiness of CA1 pyramidal cells. These data suggested that $I_M$ normally counteracts the depolarizing drive furnished by $I_{NaP}$. Hence, it is expected that $N_s$ will increase with $I_{NaP}$ and decrease with $I_M$. The situation, however, is more complicated because other states appear in the model, as we describe in the following text.

We explored the effects of varying $g_{NaP}$ and $g_M$ at a given $I_{app}$. In Fig. 8, we present maps of the various states as a function of $g_{NaP}$ and $g_{NaM}$. For brief pulses (Fig. 8A), bursting states appeared at intermediate $g_{NaM}$ and $g_{NaM}$ values that were not too small. In this bursting regime, $N_s$ increased with $g_{NaP}$ and decreased with $g_{NaM}$. Three other types of patterns appeared for $g_{NaP}$ values above the regimes of single bursts or spikes, denoted by I–III in Fig. 8A: I, a fast burst of spikes with decaying amplitudes followed by a high plateau; II, sustained, high-frequency firing; III, an irregular burst with many fast, low-amplitude spikes. The patterns in regimes I and II coexisted with the rest states, namely, the system was bistable in these parameter regimes.

The map obtained for neuronal responses to prolonged stimuli (Fig. 8B) was qualitatively similar to the previous map (Fig. 8A), although there were some notable differences. First, there were parameter regimes having $N_s = 1$ (regular spiking behavior) that markedly differed in their firing rates. The firing rate in the regime on the right side of the map, denoted by “$I_{slow}$” was 5–10 Hz, whereas the firing rate in the regime on the left side of the map, denoted by “$I_{fast}$” was in the order of 100 Hz. Second, for $g_{NaP} = 0$, it was possible to obtain a burst of two spikes in a restricted $g_M$ domain. For larger $g_{NaP}$ values, there were two “fingers” in the map where $N_s$ was 2. The finger that extended to the right showed bursting with frequency in the order of 5 Hz. A second finger extending from $g_{NaP} = 0$ to higher $g_{NaP}$ values (adjacent to the “$I_{fast}$” regime) displayed spiking at a high rate (in the order of 100 Hz) in a doublet manner: the interspike interval alternated between larger and smaller values. Between the two fingers (for $g_M$ values larger

\[ I_{app} = 0 \text{ µA/cm}^2 \]

\[ g_{M} = 0 \text{ mS/cm}^2 \]

\[ f = 0 \text{ Hz} \]
COMPARING EXPERIMENTAL RESULTS, MODELING, AND THEORY. We find that both in the experiment and in the simulations: 1) blocking $g_m$ increases the $N_s$ evoked by brief stimuli (Figs. 3A, middle and left, and 8A) and eventually transfers the neuron to a bistable tonic firing mode (Figs. 3A, third panel from left, and 8A). 2) Blocking $g_{NaP}$ after the $g_m$ blockade causes the neuron to fire only one spike in response to a brief pulse (Figs. 3A, right, and 8A). 3) In response to a prolonged pulse, blocking $g_m$ can transfer the neuron to a high plateau depolarization state (Figs. 3B, middle and left; and 8B). 4) If $g_{NaP}$ is also blocked, the neuron fires in a regular mode or becomes quiescent (Figs. 3B, right; and 8B). The conclusions of the analysis that are demonstrated in the simulations (Fig. 8) are 1) $g_m$ should be strong enough, but not too strong, to obtain bursting, 2) $N_s$ decreases with $g_{NaP}$, and 3) $f$ is proportional to $1/\tau_c$.

Note that the firing frequency of the real neuron under the effects of both linopirdine and riluzole is of order 10 Hz (Fig. 3B), which corresponds to the firing pattern denoted by $I_{fast}$ in Fig. 8B and not to $I_{fast}$. In contrast, in the cases in which the neuron bursts in “intact” cells and blocking $g_m$ leads to high plateau, additional blockade of $g_{NaP}$ in the model leads to fast tonic firing. This means that in this respect, the specific example in Fig. 3B is different from what is shown in Fig. 8B. In reality, but not in the model, the high plateau depolarization eventually decreases and the neuron repolarizes (compare Fig. 3B, middle, with Fig. 8B, I). This is because the model does not include dynamical processes with very slow time scale, such as slow inactivation of $I_{CaP}$ or of $I_{Na}$ (e.g., Fleidervish et al. 1996; French et al. 1990; Mickus et al. 1999).

Effects of varying $[Ca^{2+}]_o$.

The analyses described in the preceding text were done in conditions of $0 [Ca^{2+}]_o$. Here we use the model to interpret intriguing experimental evidence (Su et al. 2001) that reducing $[Ca^{2+}]_o$, but not blocking $Ca^{2+}$ currents and $Ca^{2+}$-activated $K^+$ currents, may transfer a nonbursting cell into a burster. Clearly raising $[Ca^{2+}]_o$ will modify the neuronal dynamics by introducing voltage-gated $Ca^{2+}$ currents and $Ca^{2+}$-activated $K^+$ currents. In addition, raising $[Ca^{2+}]_o$ will shift the voltage dependence of $I_{CaP}$ activation back toward more positive potentials (Li and Hatton 1996; Yue et al. 2005). Figure 9, A—C, illustrates the firing patterns of the neuron model obtained for three sets of parameters ($\theta_p$ is the half-maximum potential of the persistent sodium current): $g_{Ca} = 0.08 mS/cm^2$, $g_{p} = -41 mV$ (Fig. 9A), $g_{Ca} = 0.05 mS/cm^2$, $g_{p} = -44 mV$ (Fig. 9B), and $g_{Ca} = 0.02 mS/cm^2$, $g_{p} = -46 mV$ (Fig. 9C). The parameter set in Fig. 9A represents parameter values of physiological $[Ca^{2+}]_o$, whereas those in Fig. 9BC represent parameters values of reduced $[Ca^{2+}]_o$. The firing pattern of the neuron model obtained for the set $g_{Ca} = 0$, $g_{p} = -47 mV$, corresponding to $[Ca^{2+}]_o = 0$, has already been presented in Fig. 6D. It can be seen that reducing $[Ca^{2+}]_o$ augments burstiness in this model.

REDDUCING $[Ca^{2+}]_o$ IS NOT EQUIVALENT TO BLOCKING $Ca^{2+}$ CURRENTS. Maps showing $N_s$ values in bursts evoked by brief and prolonged current pulses as a function of $\theta_p$, and a second parameter related to the $Ca^{2+}$-gated currents or $Ca^{2+}$-activated $K^+$ currents, are shown in Fig. 10. Increasing $g_{Ca}$ with all $Ca^{2+}$-activated $K^+$ currents blocked ($g_{Ca} = g_{AHP} = 0$) aug-

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**Fig. 8.** Modeling A and B: dependence of $N_s$ on $g_{NaP}$ and $g_{Na}$ for $[Ca^{2+}]_o = 0$, $\tau_c = 75 ms$. Symbols are as in Fig. 7. A: values of $N_s$ in bursts evoked by brief stimuli with $I_{fast} = 7 mA/cm^2$. Interestingly, $N_s$ could switch abruptly from 1 to a value larger than 2 as $g_{NaP}$ increased. For example, for $g_{Na} = 0.8 mS/cm^2$, $N_s$ switched from 1 to 3 at $g_{NaP} = 0.23 mS/cm^2$. B: values of $N_s$ in bursts evoked by prolonged stimuli with $I_{slow} = 1 mA/cm^2$. The symbols “1fast” and “1slow” denote the value larger than 2 as $g_{NaP}$ decreases with $g_{Na}$. 0.6 mS/cm², the neuron exhibited normal bursting for intermediate $g_{NaP}$ values. Three types of patterns, analogous to the patterns obtained for brief pulses, are denoted by I–III in Fig. 8B.

To conclude, for both types of stimuli, regular bursts with large $N_s$ appear in a restricted parameter regime with intermediate values of $g_{NaP}$ and $g_{Na}$, composed of diagonal bands of constant $N_s$. In this regime, $N_s$ indeed increases with $g_{NaP}$ and decreases with $g_{Na}$. Several irregular patterns appear outside of this regime.

For a given $N_s$, $f$ generally decreased as $g_{Na}$ increased; At $g_{Na}$ values at which $N_s$ decreased by 1, $f$ increased abruptly (Fig. 8C). At and above a critical $g_{Na}$ value (3.4 mS/cm²), the neuron became quiescent ($f = 0$). As expected from the fast-slow analysis (Table 1, Eqs. 4 and 5, Fig. 5), $f$ depends linearly on $1/\tau_c$ for large $\tau_c$ (Fig. 8D). Jumps in $f$ occur at moderate values of $\tau_c$ at every $\tau_c$ value for which $N_s$ increases by 1.
transferred the system to a higher level of burstiness (for example, when g_{Ca} = 0.2 mS/cm^2 and \( \theta_p = -45 \) mV, Fig. 10H). Similarly, although blocking g_{Ca} did not lead to bursting for our reference parameter set (Fig. 9E), it could increase \( N_s \) for lower values of \( \theta_p \) (Fig. 10, D and E). For brief stimuli, bursting was obtained for more hyperpolarized values of \( \theta_p \) in comparison to the case of prolonged stimuli. Therefore evoking bursts with brief stimuli required lower values of [Ca^{2+}]_o than evoking bursts with prolonged stimuli.

COMPARING BETWEEN EXPERIMENTAL AND MODELING RESULTS. We observed both in experiments (Fig. 1) and in the simulations (Figs. 6D, 9, A-C, and 10, G and H) that reducing [Ca^{2+}]_o increases the propensity for bursting in response to brief and prolonged stimuli and augments \( N_s \). The neuron model also mimicked two more experimental results obtained in CA1 pyramidal neurons: blocking Ca^{2+} currents pharmacologically, which also blocks Ca^{2+}-activated K^+ currents, or blocking the latter currents only (by injecting a fast Ca^{2+} buffer into the neuron), did not change the regular firing mode

vented \( N_s \) for both brief and prolonged stimuli (Fig. 10, A and B) and weakly reduced \( f \) (Fig. 10C). To assess the effects of Ca^{2+}-activated K^+ conductances, we set \( g_{Ca} = 0.08 \) mS/cm^2 and computed \( N_s \) as a function of \( \theta_p \) and either \( g_{Ca} \) or \( g_{AHP} \). As expected, increasing \( g_{Ca} \) suppressed burstiness and decreased \( N_s \) for a specific value of \( \theta_p \) (Fig. 10, D and E); \( f \) increased with \( g_{Ca} \) (Fig. 10F). Increasing \( g_{AHP} \) within the range of the parameter we used (from 0 to 20 mS/cm^2) did not affect \( N_s \) substantially (data not shown). This finding is expected because the \( I_{AHP} \) is slow, and the burst is terminated by \( I_m \) before \( I_{AHP} \) becomes strong enough to have a considerable effect.

When Ca^{2+}-activated K^+ conductances were intact, the effect of varying \( g_{Ca} \) on \( N_s \) depended on \( g_{Ca} \). The value of \( N_s \) increased with \( g_{Ca} \) for small \( g_{Ca} \) values and decreased with \( g_{Ca} \) for large \( g_{Ca} \) values. Figure 10, G–I, demonstrates that with our reference parameter set (\( g_{Ca} = 10 \) mS/cm^2, \( g_{AHP} = 5 \) mS/cm^2), the hyperpolarizing effect of \( g_{Ca} \) dominated, \( N_s \) decreased with \( g_{Ca} \) for both brief and prolonged stimuli, and \( f \) increased with \( g_{Ca} \).

The increase in burstiness on reducing [Ca^{2+}]_o resulted mainly from the decrease in \( \theta_p \). However, the decrease of \( g_{Ca} \) per se also could affect \( N_s \). Although in most cases blocking \( g_{Ca} \) did not change \( N_s \) (e.g., Fig. 9D and points 9A and 9D in Fig. 10H), there were also conditions in which blocking \( g_{Ca} \)

![FIG. 9. Modeling: variant firing patterns of the neuron model for different values of [Ca^{2+}]_o. The sets of parameters \{g_{Ca} (mS/cm^2), g_{AHP} (mS/cm^2), \( \theta_p \) (mV)\} are A: [0.08, 10, 5, -41], B: [0.05, 10, 5, -44], C: [0.02, 10, 5, -46], D: [0, 10, 5, -46], E: [0.08, 0, 0, -41]. The parameter sets A–C mimic gradual reduction of [Ca^{2+}]_o from the physiological level A through B and C; when [Ca^{2+}]_o becomes 0, the parameter set \{0, 10, 5, -47\} is equal to that of Fig. 6D and is not shown here again. The parameter set D mimics blockade of Ca^{2+} conductance. The parameter set E mimics Ca^{2+} buffering, which eliminates Ca^{2+}-dependent \( K^+ \) currents. The neuron model is at rest before the current injection. Each column in the figure includes 3 voltage traces. The panels in a and b show the responses of the neuron model to prolonged strong and weak stimuli, respectively. c show the responses of the neuron model to brief (3 ms) stimuli. The horizontal dashed line represents \(-80 \) mV. The waveforms of the stimuli are provided below the voltage trace(s). Stimulus intensities are 1 \( \mu A/cm^2 \) for a, 0.7 \( \mu A/cm^2 \) for b, and 7 \( \mu A/cm^2 \) for c. Reducing [Ca^{2+}]_o (A–C), but neither blocking \( g_{Ca} \) (D) nor blocking \( g_{Ca} \) and \( g_{AHP} \) (E), induces bursting in a regular firing cell.

![FIG. 10. Modeling. Top and middle: dependence of \( N_s \) on either \( g_{Ca} \) or \( g_{Ca} \) and \( \theta_p \). Symbols are as in Fig. 7. Top: response to a brief pulse with \( I_m = 7 \mu A/cm^2 \). Middle: response to a prolonged pulse with \( I_{AHP} = 1 \mu A/cm^2 \). Bottom: dependence of the bursting frequency \( f \) on either \( g_{Ca} \) or \( g_{Ca} \) for \( I_m = 7 \mu A/cm^2 \) and \( \theta_p = -46 \) mV. A and B, \( N_s \) vs. \( g_{Ca} \) and \( \theta_p \); C and D, \( N_s \) vs. \( g_{Ca} \) and \( g_{AHP} \); E and F, \( N_s \) vs. \( g_{Ca} \) and \( g_{AHP} \) plane for \( g_{Ca} \) = 0.08 mS/cm^2; \( g_{AHP} = 5 \) mS/cm^2; \( g_{AHP} = 0 \). C and D, \( f \) vs. \( g_{Ca} \) for \( g_{Ca} = g_{AHP} = 0 \). D and E, \( N_s \) vs. \( g_{Ca} \) and \( \theta_p \) plane for \( g_{Ca} \) = 0.08 mS/cm^2, \( g_{AHP} = 0 \). G and H: \( N_s \) vs. \( g_{Ca} \) and \( \theta_p \) for \( g_{Ca} = 10 \) mS/cm^2, \( g_{AHP} = 5 \) mS/cm^2. I: \( f \) vs. \( g_{Ca} \) for \( g_{Ca} = 10 \) mS/cm^2, \( g_{AHP} = 5 \) mS/cm^2. In all the panels, \( N_s \) increases as \( \theta_p \) is hyperpolarized. The solid circles in panels E, G, and H denoted by "9" or "6" and letters, such as "9A," indicate the corresponding column in Figs. 9 or 6, respectively.

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of these neurons (Su et al. 2001). Examples are shown in Fig. 9D, in which \( g_{Ca} \) is set to 0 (other parameters, including \( \theta_h \), are as in Fig. 9A), and in Fig. 9E, where both \( g_R \) and \( g_{AHP} \) are set to 0. It can be seen in these figures that blocking \( Ca^{2+} \)-activated \( K^+ \) currents in either way does not modify the firing behavior of the neuron model for the chosen parameter set.

**DISCUSSION**

**Major conclusions**

Our finding that truncated neurons fire in a similar manner to intact neurons in both normal and low \([Ca^{2+}]_o\), allowed us to use a single-compartment model to analyze the underlying mechanism of bursting. Our analysis leads to several conclusions: first, CA1 pyramidal cells can burst via a single-compartment mechanism with one slow variable, namely the activation variable of \( I_M \) (\( N \)), provided two conditions are fulfilled, 1) The kinetics of the spike generating currents (i.e., \( I_Na \) and \( I_Ka \)) are fast, although not too fast and 2) the activation curve of \( I_M \) is properly tuned. If it is too depolarized, the neuron will fire tonically; if it is too hyperpolarized, the neuron will be quiescent. Accordingly, it was shown experimentally that shifting the activation curve of \( I_M \) toward more negative potentials with retigabine (Tatulian et al. 2001; Wickenden et al. 2000) suppresses bursting behavior in both normal and 0 \([Ca^{2+}]_o\) (Yue and Yaari 2004).

Second, moderate values of \( g_{NaP} \) favor burstiness because it enhances bistability of the fast subsystem. Thus \( N_S \) increases with \( g_{NaP} \). Assuming a natural heterogeneity in \( g_{NaP} \), the model can replicate the diversity of intrinsic firing patterns found experimentally (Fig. 6), but if \( g_{NaP} \) is too large, the neuron will display prolonged bursts with many low-amplitude spikes or a high plateau depolarization (Figs. 7 and 8). Bistability and bursting, however, can be supported also by the window \( I_Na \) provided it is sufficiently large (not shown). Therefore the presence of \( g_{NaP} \) is not a necessary condition for bursting in our model.

Third, \( g_M \) is mandatory for bursting in 0 \([Ca^{2+}]_o\). This feature of the model is consistent with our experimental findings showing that blocking \( g_M \) with linopirdine converts bursting in 0 \([Ca^{2+}]_o\) to plateau depolarization (Fig. 3). Conversely, increasing \( g_M \) decreases \( N_S \). Ultimately, when \( g_M \) is large enough, the neuron does not fire spikes. When \([Ca^{2+}]_o\) is elevated, \( I_{AHP} \) and \( I_M \) play a similar role in the neuronal dynamics, with \( I_{AHP} \) being slower.

Fourth, our model predicts that shifting the activation curve of \( I_{NaP} \) to more negative potentials will increase burstiness (Fig. 10). Given that such a shift is obtained by lowering \([Ca^{2+}]_o\) (Li and Hatton 1996; Yue et al. 2005), the model readily accounts for the experimental finding that lowering \([Ca^{2+}]_o\), but not blocking \( Ca^{2+} \) and \( Ca^{2+} \)-activated conductances, induces bursting behavior in regular spiking CA1 pyramidal cells (Su et al. 2001).

**Comparison between experimental and modeling results**

The dynamics of the neuron model have been compared with the dynamics of native neurons from several aspects.

1. **Can we obtain in the model the variety of firing patterns recorded experimentally?** We have shown in Figs. 6 and 4B that by varying \( g_{NaP} \) and \( V_L \), the model can mimic the various firing parameters obtained in \([Ca^{2+}]_o = 0\) (see Fig. 1 in Su et al. 2001). In reality, there is biological heterogeneity in other parameters as well that may contribute to the variety of firing patterns. Similarly, the model can mimic the firing patterns obtained experimentally at various levels of \([Ca^{2+}]_o\), \( g_{Cat} \), and \( g_C \) (compare Fig. 9 with Fig. 1 here and Fig. 2 in Sue et al. 2001).

2. **How does the propensity for bursting and \( N_S \) vary with \( I_{app} \), \( g_{NaP} \), and \( g_C \)?** In the neuron model (Fig. 7) as well as in native neurons (Fig. 2), \( N_S \) increases with \( g_{NaP} \) and depends only weakly on \( I_{app} \). When the neuron bursts, \( N_S \) decreases with \( g_M \) (Fig. 8) (see Fig. 1 in Yue and Yaari 2004) and reaches a fast tonic firing state or a high plateau for low levels of \( g_M \) (Figs. 3 and 8).

3. **How does the propensity for bursting and \( N_S \) vary with \( g_{Ca} \), \( g_C \), and \( [Ca^{2+}]_o \)?** It has been reported that reducing \([Ca^{2+}]_o\), but not blocking the \( Ca^{2+} \) current or \( Ca^{2+} \)-activated \( K^+ \) currents, increases the propensity for bursting and \( N_S \) (see Figs. 3, 7, and 8 in Sue et al. 2001). This experimental observation can be replicated in the model (Fig. 10) using certain parameter sets and the assumption that elevating \([Ca^{2+}]_o\) causes \( \theta_h \) to shift to more positive voltage. Figure 10 suggests that in principle, however, there may be cases in which \( N_S \) increases with the blockade of \( g_C \) (Fig. 10, D and E) or \( g_{ca} \) (Fig. 10, G and H).

**Discrepancies between modeling and experimental results.**

The dynamical equations of the model do not include slow processes (>1 s). Therefore the model cannot explain slow changes observed experimentally, such as the slow repolarization of the membrane potential during a high plateau and the resumption of spiking (Fig. 3, middle).

In this paper, we show several diagrams in which \( N_S \) varies as a function of two parameters (Figs. 7, 8, and 10). When a third parameter is varied, the relative positions of the areas of particular \( N_S \) values may shift. The two-dimensional diagrams provide therefore qualitative information about the type of transition that may occur when parameters vary, but one cannot expect that the experimental behavior will always follow the two-dimensional diagrams quantitatively. For example, Fig. 8B shows that in response to a prolonged pulse, reducing \( g_M \) may transfer a bursting pattern to a high plateau, and further reduction of \( g_{NaP} \) may transfer a high plateau neuron to a neuron spiking at high rates. Figure 8B does not overlap the example of Fig. 3, in which \( g_M \) blockade by linopirdine followed by \( g_{NaP} \) blockade by riluzole transfers a bursting pattern to a high plateau and then to a tonic firing pattern (with firing frequency of ~10 Hz). It is possible, however, that linopirdine and riluzole, used to blocked \( g_M \) and \( g_{NaP} \), respectively, exert additional effects on neuronal excitability. Note that Fig. 3A is consistent with Fig. 8A regarding the effects of blocking \( g_M \) and \( g_{NaP} \) on the firing patterns in response to a brief current pulse. In both cases, blocking \( g_M \) in the case of bursting neurons can bring the neuron to a fast spiking state leading to a high plateau, and subsequent blockade of \( g_{NaP} \) leads to firing of one spike only.

For the model, we chose a \( \theta_h \) value that is ~20 mV depolarized compared with available voltage-clamp experimental measurements (Colbert and Pan 2002; Gasparini and Magee 2002; Martina and Jonas 1997), to obtain a large enough window \( I_Na \) to support bistability in the fast subsystem.
Window $I_{Na}$ is needed for bursting and even for spiking in previous models as well and is obtained by assuming a small value of $\theta_m - \theta_z$ (e.g., 4.2 mV in Pinsky and Rinzel 1994; Traub and Miles 1991; Traub et al. 1991) or by assuming a very large $8_{Na}$ value in the axo-somatic compartment (Mainen and Sejnowski 1996).

**Comparison with other models of bursting neurons**

A 16-compartmental model of CA1 pyramidal cells, albeit with passive dendrites, was provided by Shuai et al. (2003). This model also displayed rhythmic bursting in zero $[Ca^{2+}]_o$. The reversal potential of the leak current in the soma was $-58$ mV, more depolarized than the value we used ($-70$ mV). The reversal potential of the leak current in the dendrites, however, was assumed to be 0 mV (Eq. 2 in Shuai et al. 2003), without any apparent biological reason. Simulating their model (using parameters of Table 1, Shuai et al. 2003), we found that after disconnecting the soma from the apical dendrite their respective resting potentials were $-63.9$ and 0 mV. Bursting in the intact neuron model depended on depolarization of the soma by the dendrite and disappeared after disconnecting the soma from the dendritic compartments. This behavior of the latter neuron model is incongruent with our experimental data showing that bursting behavior persists in truncated neurons (Figs. 1, 2, 3, and 4A).

The kinetics of ionic currents in the model of Shuai et al. (2003) also differed considerably from ours. Most notably, the activation time constant of $I_{NaP}$ in their model was 30 ms even though experimental measurements have shown it to be well below 1 ms (Kay et al. 1998; Magistretti et al. 2003; Vervaeke et al. 2006). In contrast, $I_{NaP}$ activation kinetics in our model is fast, and $z$ is the only slow variable. Consequently, bistability of the fast subsystem of variables is needed to generate bursting behavior.

In other models of hippocampal and neocortical pyramidal neurons, intrinsic bursting in normal $[Ca^{2+}]_o$ was attributed to a ping-pong interplay between fast Na$^+$ and K$^+$ currents in the soma and slow Ca$^{2+}$ and K$^+$ (mostly Ca$^{2+}$-dependent) currents in the apical dendrites (Bazhenov et al. 2004; Mainen and Sejnowski 1996; Pinsky and Rinzel 1994; Traub and Miles 1991; Traub et al. 1991). The latter mechanism depends on the coupling between soma and apical dendrites; bursting behavior was not obtained when coupling resistance was too small or too large.

The CA1 pyramidal cell has widely branched basal dendrites, which are electrotonically compact (Zador et al. 1995). In general, the electrotonic separation between the axon, the soma and the basal dendrite may modify the firing patterns of the neuron. The existence of a slow current, such as $I_M$, and bistability of the fast subsystem, will be needed for bursting even in models similar to ours where the spatial structure of the truncated dendrite is explicitly considered. Effects of the electrotonic separation between soma and axon can be addressed theoretically as more data about the axon becomes available.

**Predictions for future experiments**

The major results and predictions of the model, such as the increase of $N_S$ with $8_{NaP}$ and its decrease with $8_M$ and the generation of high plateaus, have been confirmed experimentally as reported here and elsewhere (Gilles et al. 1999; Su et al. 2001; Yue and Yaari 2004, 2006). The model yields several more predictions. For example, increasing $8_{NaP}$ ultimately will lead to prolonged bursts with many low-amplitude spikes (Fig. 8) or to high-plateau depolarization. Also, increasing $8_{Ca}$ in normal $[Ca^{2+}]_o$ will moderately decrease the propensity for bursting when $Ca^{2+}$-activated K$^+$ currents are operative and strong enough and will moderately increase this propensity when these currents are blocked (Figs. 9 and 10). It would be interesting to test these predictions by modulating these various currents pharmacologically or by simulating changes in these currents using the dynamic-clamp technique (Sharp et al. 1993; Vervaeke et al. 2006).

**Functional implications**

Our experimental and computational results suggest that bursting in adult CA1 pyramidal cells in zero and in low $[Ca^{2+}]_o$, in vitro is generated by a “square-wave” mechanism, i.e., from interplay between ionic currents located within one compartment, namely, the soma/axon initial segment. Thus separation between fast currents in the soma/axon initial segment and slow currents in the apical dendrites (the ping-pong mechanism) is not obligatory for bursting. However, the latter mechanism may be invoked in adult CA1 pyramidal cells by blocking $I_s$ pharmacologically after which backpropagating spikes evoke dendritic Ca$^{2+}$ spikes that, in turn, trigger somatic bursting (Magee and Carruth 1999). A similar ping-pong mechanism may exist naturally in developing CA1 pyramidal cells (Chen et al. 2005). Thus CA1 pyramidal cells may display both square-wave and ping-pong bursting, depending on their developmental stage and on the composition of the extracellular milieu. Interestingly, although it has been established that pyramidal neurons in cortical layer V can display a ping-pong bursting behavior in some conditions (Larkum et al. 1999), they can also burst after most of their apical dendrites are truncated (A. E. Telfeian and B. W. Connors, personal communication). Thus the square-wave mechanism described here for CA1 pyramidal cells may be relevant for those neurons also.

Hippocampal pyramidal cells in vivo were shown to alternate between regular firing (“simple” spikes) and burst firing (“complex” spikes), depending on the behavioral state of the animal (Ranck 1973). It is not yet known how intrinsic factors contribute to complex spike bursting in vivo. However, decreases in $[Ca^{2+}]_o$, which occur during synchronized neuronal activity (Heinemann et al. 1977) and may attain levels of ≤0.2 mM during epileptic seizures (Pumain et al. 1985), are expected to induce square-wave bursting in CA1 pyramidal cells in vivo. Likewise, factors that upmodulate $8_{NaP}$ (e.g., hypoxia or the accumulation of nitric oxide) (Hammarstrom and Gage 2002), or downmodulate $8_M$ (e.g., a variety of neurotransmitters) (Brown and Yu 2000), also are likely to enhance this mode of bursting in these neurons in vivo.

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SQUARE-WAVE BURSTING IN CA1 PYRAMIDAL CELLS


