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Contribution of persistent Na\(^+\) current and M-type K\(^+\) current to somatic bursting in CA1 pyramidal cells: combined experimental and modeling study

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Running head: Square wave bursting in CA1 pyramidal cells
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ABSTRACT

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\(^{2+}\)]\(_o\), though not by blocking Ca\(^{2+}\) and Ca\(^{2+}\)-activated K\(^+\) currents. We show experimentally using intracellular recordings that burstiness in low [Ca\(^{2+}\)]\(_o\) 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 model of CA1 pyramidal neurons. In this neuron model, reduced [Ca\(^{2+}\)]\(_o\) is simulated by negatively shifting the activation curve of the persistent Na\(^+\) current (\(I_{\text{NaP}}\)), 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 \(I_{\text{NaP}}\) 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, though 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 \(I_M\).
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 (Schwartzkroin, 1975; Jensen et al., 1994; 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 (Jensen et al., 1994; Azouz et al., 1996; 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 (Lisman, 1997; Yaari and Beck, 2002; Izhikevich et al., 2003), 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 (Wong and Prince, 1981; Larkum et al., 1999; Jung et al., 2001; Chen et al. 2005; Metz et al. 2005). 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 (Pinsky and Rinzel, 1994; Warman et al. 1994; Traub and Miles, 1991; Traub et al. 1991, 1994; Mainen and Sejnowski, 1996). 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 than the 'ping-pong' mechanism which depends on the integrity of apical dendrites.
We study the mechanism by which changes in \([\text{Ca}^{2+}]_o\) modulate transitions between regular firing and bursting in adult CA1 pyramidal cell by combining electrophysiological, computational and analysis techniques.

**MATERIALS AND 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 anesthesia, and transverse hippocampal slices (400 μm) were prepared with a vibrating microslicer (Leica, Germany) and transferred to a storage chamber perfused with oxygenated (95% O₂ - 5% CO₂) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 3.5, MgCl₂ 2, CaCl₂ 1.6, NaHCO₃ 26 and D-glucose 10, pH 7.4, osmolarity 305 mOsm, where they were maintained at room temperature. Slices were placed one at a time in an interface chamber (33.5° C) and perfused with oxygenated ACSF. In order to truncate the apical dendrites of CA1 pyramidal cells, a deep cut was made in stratum radiatum close to and parallel to stratum pyramidale using a broken pipette or a razor blade chip propelled by a micromanipulator (Yue et al. 2005). Truncation was confirmed by recording field potentials in stratum pyramidale (see below) before and after cutting. Orthodromic field potentials evoked by stimulating in stratum radiatum disappeared after cutting, while antidromic field potentials evoked by stimulating in alveus remained large (Yue et al. 2005). The slices were allowed to recover in the chamber for at least 1 hour before initiating a recording session.

**Electrophysiological recordings**

Intracellular recordings were obtained using sharp glass microelectrodes containing 4 M K⁺-acetate (90-110 MΩ). An active bridge circuit in the amplifier (Axoclamp 2B, Axon Instruments, Foster City, CA) allowed simultaneous injection of current and measurement of membrane potential. The bridge balance was carefully monitored and adjusted before each measurement. The intracellular signals were filtered on-line at 10 kHz, digitized at a sampling rate of 10 kHz or more, and stored by a personal computer using a data acquisition system (Digidata 1322A) and pCLAMP software (Axon Instruments).
Drugs

Stock solutions of 4β-phorbol 12,13-dibutyrate (PDB; 10 mM), riluzole (10 mM) and phenytoin (100 mM) were prepared in dimethyl sulfoxide (DMSO) and stored at -20° C. They were usually diluted at 1:1000 when added to the ACSFs. Control ACSFs contained equal amounts of DMSO (0.001%), which by itself had no effects on the measured parameters. All other drugs were added to the ACSFs from aqueous stock solutions. Chemicals and drugs were obtained from Sigma (Petach-Tikva, Israel).

Cell model

The somatic, single compartment model was represented by coupled differential equations according to the Hodgkin-Huxley-type scheme. We constructed the model in two stages. In the first stage, we introduced only the ionic currents that are involved in firing dynamics in zero [Ca\(^{2+}\)]\(_o\), at which it is simpler to analyze. In the second state, we added voltage-gated Ca\(^{2+}\) and Ca\(^{2+}\)-activated K\(^+\) currents, to explore their influence on bursting behavior.

Model for zero [Ca\(^{2+}\)]\(_o\). The model includes the currents that are known to exist in the soma and proximal dendrites: the transient Na\(^+\) current (\(I_{Na}\)) and the delayed rectifier K\(^+\) current (\(I_{Kdr}\)) that generate spikes, and the muscarinic sensitive K\(^+\) current (\(I_{M}\)) that contributes the slow variable necessary for bursting (Bertram et al. 1995; Yue and Yaari, 2004, 2006). A model with these three currents only is the minimal model that allows bursting. We added the persistent sodium current (\(I_{NaP}\)) because we wanted to focus on its contribution to bursting (Su et al. 2001; Yue et al. 2005). The A-type K\(^+\) current (\(I_A\)) is included as well even though its density is much higher in the apical dendrites than in the soma (Hoffman et al. 1997). The current balance equation is (Borg-Graham, 1999)

\[
C \frac{dV}{dt} = -g_L(V - V_L) - I_{Na} - I_{NaP} - I_{Kdr} - I_A - I_M + I_{app}
\]

where \(C = 1 \ \mu F/cm^2\), \(g_L = 0.05 \ mS/cm^2\), \(V_L = -70 \ mV\), and \(I_{app}\) is the applied current. The ionic currents are:

\[
I_{Na} (V,h) = g_{Na} m^3 (V) h (V - V_{Na}), \quad I_{NaP} (V) = g_{NaP} P_{\infty} (V) (V - V_{Na}),
\]

\[
I_{Kdr} (V,n) = g_{Kdr} h^4 (V - V_K), \quad I_A (V,b) = g_A a^3 (V) b (V - V_K), \quad I_M (V,z) = g_M z (V - V_K).
\]
conductances and reversal potentials are: \( g_{Na} = 35 \) mS/cm\(^2\), \( g_{NaP} \) varies between 0 and 0.41 mS/cm\(^2\), \( g_{Kd} = 6 \) mS/cm\(^2\), \( g_{A} = 1.4 \) mS/cm\(^2\), \( g_{M} = 1 \) mS/cm\(^2\), \( V_{Na} = 55 \) mV, \( V_{K} = -90 \) mV. The kinetics equations and parameters are listed in Table 1. The cell model for zero \([Ca^{2+}]_o\) has 5 dynamical variables: \( V, h, n, b \) and \( z \).

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

\[
C \frac{dV}{dt} = -g_L(V - V_L) - I_{Na} - I_{NaP} - I_{Kd} - I_A - I_M - I_{Ca} - I_C - I_{sAHP} + I_{app} \tag{2}
\]

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

\[
\frac{d[Ca^{2+}]}{dt} = -\nu I_{Ca} - [Ca^{2+}]_i / \tau_{Ca} \tag{3}
\]

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

The low threshold T-type \( Ca^{2+} \) current was not included in the model, because it is localized mostly in the distal apical dendrites of adult CA1 pyramidal cells (Thompson and Wong, 1991; Karst et al. 1993). The hyperpolarization-activated cationic current \( (I_h) \) (Magee, 1998, Vasilyev 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 $I_h$ are faster
than that of $I_M$ (Macciaferri 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) Ca$^{2+}$-activated K$^+$ current ($I_{AHP}$), which can be evoked under voltage-clamp
conditions in CA1 pyramidal cells (Stocker et al., 1999; Gu et al., 2005), 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, pulse duration was very long, allowing
neuronal dynamics to reach steady-state behavior (mathematically, converging to an attractor). There
were also conditions in which the neuron model fired spontaneously without application of positive
currents.

**Firing patterns.** Depending on the model parameters, the neuron model might fire in one of
two patterns when stimulated with prolonged positive current pulses: (i) regular, tonic firing, in
which neurons fired solitary spikes in a periodic manner. Often, two distinct types of tonic firing
were observed: low-frequency tonic firing at frequencies of up to ~5 Hz; and high-frequency tonic
firing, in which neurons fired continuously at ~100 Hz. (ii) Rhythmic bursting, in which sequential
bursts were separated by periods of neuronal quiescence, typically lasting several hundreds of
milliseconds. Rhythmic bursting was either periodic or aperiodic (chaotic) (Terman, 1992).

**Bursting.** In this study we defined a burst as a tight cluster (inter-spike interval in the order of
10 ms) of two or more spikes generated alone (as in the case of bursts evoked by brief stimuli) or
distinctly separated from the following spikes (as in the cases of spontaneous bursting or bursting
evoked by prolonged stimuli). In the cases of prolonged or spontaneous activity, we quantified
bursting behavior at long times (after the dynamics has converges to an attractor), namely after transient effects of the initial conditions have decayed. The average number of intraburst spikes \( (N_S) \) was computed by averaging the number of spikes per burst during a 1.5 s period 1 s after stimulus onset (i.e. after the dynamics has converged to an attractor), and rounding up this average to the closest integer. Doublet states were particular cases of periodic bursting states in which the neuron model fired exactly two spikes in a burst, namely, \( N_S = 2 \) (Mandelblat et al. 2001). Clearly, in regular spiking cells \( N_S = 1 \).

**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 \( I_{NaP} \) 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 \([Ca^{2+}]_o = 0\) (and thus \( \theta_p = -47 \) mV and \( g_{Ca} = 0\)) 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 5 dynamic variables: \( V, h, n, b \) and \( z \). The first 4 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 \( \tau_z \to \infty \), where \( \tau_z \) is the time constant of \( z \). It describes an approximation of the dynamics for large but finite \( \tau_z \). 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), \( V \) is determined by its value for the fixed point of the fast subsystem for the instantaneous value of \( z \), denoted by \( V_{rest, fast}(z) \), whereas \( z \) evolves slowly (Table 1). If \( z_{<} \)(\( V_{rest, fast}(z) \)) > \( z \), \( z \) increases slowly, and if \( z_{>} \)(\( V_{rest, fast}(z) \)) < \( z \), \( z \) decreases slowly. If, for a certain value of \( z \) denoted by \( z_{FP} \), \( z_{>} \)(\( V_{rest, fast}(z_{FP}) \)) = \( z_{FP} \), namely, the line representing the stable rest state, \( V_{rest, fast}(z) \), intersects with \( z_{<}(V) \), the activation curve of \( z \) (Table 1), the point \((V_{rest, fast}(z_{FP}), z_{FP})\) represents a fixed point of the full system. It is stable if \( dV_{rest, fast}(z) / dz < [dz_{<}(V) / dV]^{-1} \). The dynamics of the fast subsystem may
converge also to a stable tonic firing state (limit cycle), where \( T_z \) is the time period of the cycle that depends on \( z \). The approximation that \( \tau_z \) is much larger than the other time scales in the system, and, specifically, \( \tau_z > T_z \), 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, Mandelblat et al. 2001, Izhikevich 2006)

\[
\left\langle \frac{dz}{dt} \right\rangle = \left[ z_{\infty}(V_{\text{equiv}}) - z \right] / \tau_z
\]

(4)

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

\[
z_{\infty}(V_{\text{equiv}}) = \frac{1}{T_z} \int_0^{T_z} z_{\infty}(V(t)) \, dt
\]

(5)

In the slow time scale, we can replace \( \left\langle dz/dt \right\rangle \) by \( dz/dt \). Therefore, the dynamics of the slow variable \( z \) is determined, on average, by the right hand side of Eq. 5. If \( z_{\infty}(V_{\text{equiv}}(z)) > z \), \( z \) increases slowly, and if \( z_{\infty}(V_{\text{equiv}}(z)) < z \), \( z \) 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 \( z, z_{\infty}(V) \), namely \( z_{\infty}(V_{\text{equiv}}(z_{\text{LC}})) = z_{\text{LC}} \). 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 \( dV_{\text{equiv}}(z)/dz < [dz_{\infty}(V)/dV]^{-1} \). 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, leftmost panel). The mean (± SEM) resting potential, apparent input resistance and spike amplitude of these neurons were -67.8 ± 0.5 mV (range -75 to -61 mV), 40.1 ± 2.0 MΩ (range, 20.5 to 48.7 MΩ) and 92.1 ± 5.7 mV (range 71.7 to 103.2 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, *leftmost column*). Like in intact neurons (Su et al. 2001; Yue et al. 2005), approximately half of the neurons were nonbursters and the other half were mostly high-threshold bursters (bursting only in response to long depolarizing current pulses of 2-3 × threshold intensity) with a small subset of low-threshold bursters (bursting in response to threshold-straddling stimuli).

In all 24 truncated neurons examined, lowering [Ca\(^{2+}\)]\(_o\) from 2 to nominally 0 mM caused a progressive increase in the spike ADP. In 19 of these neurons (79.2%) the increase in spike ADP culminated in a high-frequency burst of 3 to 6 spikes as their minimal response to threshold depolarization (Fig. 1A). Lowering [Ca\(^{2+}\)]\(_o\) also reduced spike frequency adaptation (Fig. 1B). These effects are similar to those described previously in intact neurons (Azouz et al. 1996; Su et al. 2001). Thus, functional apical dendrites are not required for the expression of bursting behavior in low [Ca\(^{2+}\)]\(_o\).

In nominally Ca\(^{2+}\)-free ACSF, many of the neurons converted to bursting mode manifested rhythmic bursting at their native resting potential (see below, Fig. 4A) or at more depolarized potentials. Increasing the depolarizing current intensity enhanced the frequency of bursting without strongly affecting the shape of individual bursts. However, at the higher frequencies of bursting, the number of intraburst spikes progressively decreased with time (Fig. 1B, *rightmost column*).

**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). In order 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 5 such neurons. Riluzole (10 µM) blocks I\(_{NaP}\) in CA1 pyramidal cells almost completely, while exerting a lesser effect on the transient Na\(^+\) current (Urbani et al. 2000; Spadoni et al. 2002; Yue et al. 2005). Representative results are shown in Figure 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 \(I_{NaP}\) \(\text{(Chao and Alzheimer, 1995; Cantrell et al. 1996; Yue et al. 2005)}\). 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 \(\text{(in the order of seconds; French et al. 1990)}\), 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 \(\text{(Yue and Yaari, 2004)}\). We tested this notion using linopirdine and XE991, selective blockers of \(I_M\) \(\text{(Schnee and Brown, 1998; Wang et al., 1998)}\). Representative results from one truncated neuron bathed in \(Ca^{2+}\)-free ACSF are shown in Figure 3. When activated by brief depolarizing pulses, the neuron generated a burst of 3 spikes \(\text{(Fig. 3A, leftmost panel)}\). 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 \(\text{(Fig. 3A, middle panels)}\). Both the primary burst and the underlying plateau potentials were readily suppressed by subsequent addition of 10 \(µM\) riluzole to the ACSF \(\text{(Fig. 3A, rightmost panel)}\), 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 \(\text{(Fig. 3B, leftmost panels)}\). Under the influence of linopirdine, rhythmic bursting converted to a plateau depolarization which lasted throughout the period of stimulation \(\text{(Fig. 3B, middle panels)}\). Again, adding 10 \(µM\) riluzole to the ACSF suppressed the initial burst responses and the plateau depolarizations and imposed a regular firing mode \(\text{(Fig. 3B, rightmost panels)}\). Similar results were obtained in 5 truncated neurons treated with 10 \(µM\) linopirdine and 2 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. Firstly, can the model account for the variant firing patterns observed experimentally as its parameters are varied? Secondly, do the propensity 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 ms) and that of bursting (about 100 ms). Experimentally, we found that many CA1 pyramidal cells also exhibited very slow dynamics (more than 1 s), that may cause rhythmic bursting to change eventually to repetitive spikes (Figs. 1B, middle column; 3B, left panel, two upper 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 above 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 \( p_3(V) \). 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.

In order 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 rest state is stable again for negative values of \( z \) (negative \( z \) values do not have physiological meaning, but they are important for a complete mathematical analysis). At the \( z \) value where the high rest state gains its stability (a Hopf bifurcation that is out of the scale in Fig. 5AB), an oscillatory state (limit cycle) emerges, corresponding to tonic, periodic firing. This oscillatory state extends toward the right. For \( g_{NaP} = 0 \) (A), 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 \( g_{NaP} = 0.2 \) mS/cm\(^2\) (B), the bistable regime,
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 \( g_{NaP} = 0 \) \((\text{Fig. 5A})\), the bistable regime is tiny and cannot generate bursting states with the present kinetics of \( z \); the neuron fires tonically. For \( g_{NaP} = 0.2 \text{ mS/cm}^2 \) \((\text{Fig. 5B})\), there is a significant bistable regime. When the neuron fires, \( z \) slowly increase 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 \( g_{NaP} = 0.3 \text{ mS/cm}^2 \) \((\text{Fig. 5C})\), except that the number of spikes \((N_z)\) 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 \( g_{NaP} = 0.41 \text{ mS/cm}^2 \) \((\text{Fig. 5D})\) 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 below, \(\text{Fig. 8BIII} \)).

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

I. 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 \([g_{Na}n^3(V)h_z(V) + g_{NaP}p_z(V)](V - V_{Na})\) 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 that 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.

**II. 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 (towards larger values of $z$) than the rest state. Hence, the bistable regime expands as $g_{NaP}$ increases. This regime may even be produced by increasing $g_{NaP}$ if it had not existed for $g_{NaP} = 0$. This means that elevating $g_{NaP}$ may induce bursting behavior and increase $N_S$. Increasing $g_{NaP}$ also shifts the Hopf bifurcation of the depolarized plateau potential to the right (compare Fig. 5 A-D). Therefore, when $g_{NaP}$ is very large, the active phase of the burst becomes a high plateau depolarization or a sequence of low amplitude fast spikes converging to a high plateau depolarization, rather than full-blown spikes (Fig. 5D).

**III. Activation curve of $I_M$.** To allow bursting behavior the activation curve of $I_M$, $z_{\alpha}(V)$, should be located between the rest state curve and the curve of $V_{equiv}$ (Bertram et al. 1995). If $z_{\alpha}(V)$ is shifted towards more depolarized levels, ($\theta_z$, the half-maximum potential of $I_M$, increases), the neuron will fire tonically. If $z_{\alpha}(V)$ is shifted towards more hyperpolarized levels ($\theta_z$ decreases), the neuron will be quiescent at rest.

**IV. Strength of $g_M$.** $I_M$ is proportional to $g_M \times z$. For $g_M = 0$ (equivalent to $z = 0$ in Fig. 5A-D), the neuron fires tonically or reaches a high plateau depolarization when $g_{NaP}$ is very large. Increasing $g_M$ is equivalent to compressing the bifurcation diagram of the fast subsystem along the $z$ axis in Figure 5, without modifying the kinetics of the slow variable $z$. Therefore, as $g_M$ increases, the slow variable $z$ spends less time in the oscillatory state and $N_S$ decreases. When $N_S$ decreases to 1 the neuron fires in a tonic pattern. For very large $g_M$, the rest state of the fast subsystem intersects with the activation curve of $z$, and the neuron becomes quiescent at rest.

**Summary of necessary conditions for bursting.** Our analysis reveals four necessary conditions for bursting. All these conditions demand that certain parameters will be in particular ranges. (i) The
kinetics of the variables underlying spike generation (inactivation of $I_{Na}$ and activation of $I_{Kdr}$) should be fast enough (otherwise, there will be no bistability and the neuron will fire continuously), but not be too fast (to prevent a plateau depolarization). 

(ii) The fast subsystem should show bistability, and $g_{NaP}$ supports bistability. Therefore, $g_{NaP}$ should be strong enough to generate bistability, unless this bistability has already been generated by the window $I_{Na}$. If $g_{NaP}$ is too strong, however, the neuron model will manifest very fast spiking with small amplitude or a plateau depolarization. 

(iii) The half-maximum potential of $I_{M}$, $\theta_z$, should not be too depolarized (at which condition the neuron will fire tonically) or too hyperpolarized (at which condition the neuron will be at resting potential). 

(iv) $g_M$ should be strong enough (otherwise, the neuron will fire in a regular mode or will display a high plateau depolarization) but not too strong (otherwise, the neuron will fire in a regular mode or will not fire at all).

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 ($\tau_z = 75$ 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.

**Effects of varying $g_{NaP}$**

CA1 pyramidal cells perfused with Ca$^{2+}$-free ACSF display a diversity of firing patterns, ranging from regular firing to spontaneous rhythmic bursting (Figs. 1-3,4A) (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 $\theta_p = -47$ mV and examined the consequences of increasing $g_{NaP}$. When $g_{NaP}$ was set to zero, the neuron fired in a regular mode during sustained depolarization (Fig. 6A). As $g_{NaP}$ was raised, the propensity for bursting increased, so that at $g_{NaP} = 0.08$ mS/cm$^2$ the neuron became a periodic burster when strongly depolarized (Fig. 6B) and at $g_{NaP} = 0.18$ mS/cm$^2$ it burst-fired periodically in response to all suprathreshold stimuli (Fig. 6C). Further increasing $g_{NaP}$ 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 $V_L$ from -70 to -62 mV destabilized the rest state and caused spontaneous rhythmic bursting when $g_{NaP}$ was high (Fig. 4B). For $g_{NaP} = 0$, the
neuron was quiescent even for \( V_L = -62 \) mV.

In all the cases shown in Figures 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.

In order 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 (top-right of Fig. 7A). 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 1 or 2 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. 7C,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. 6,7) 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 below.

We explored the effects of varying $g_{NaP}$ and $g_M$ at a given $I_{app}$. In Figure 8 we present maps of the various states as a function of $g_M$ and $g_{NaP}$. For brief pulses (Fig. 8A), bursting states appeared at intermediate $g_{NaP}$ and $g_M$ values that were not too small. In this bursting regime, $N_S$ increased with $g_{NaP}$ and decreased with $g_M$. Three other types of patterns appeared for $g_{NaP}$ values above the regimes of single bursts or spikes, denoted by I-III in Figure 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), though there were some notable differences. First, there were parameter regimes having $N_S=1$ (regular spiking behavior) which markedly differed in their firing rates. The firing rate in the regime on the right side of the map, denoted by "1slow", was 5-10 Hz, whereas the firing rate in the regime on the left side of the map, denoted by "1fast", was in the order 100 Hz. Secondly, for $g_{NaP}=0$, it was possible to obtain a burst of 2 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 "1fast" 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 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 Figure 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 $g_M$, composed of diagonal bands of constant
$N_S$. In this regime, $N_S$ indeed increases with $g_{NaP}$ and decreases with $g_M$. Several irregular patterns appear outside of this regime.

For a given $N_S$, $f$ generally decreased as $g_M$ increased; At $g_M$ values at which $N_S$ decreased by 1, $f$ increased abruptly (Fig. 8C). At and above a critical $g_M$ value (3.4 mS/cm$^2$), the neuron became quiescent ($f = 0$). As expected from the fast-slow analysis (Table 1, Eqs. 4, 5, Fig. 5), $f$ depends linearly on $1/\tau_z$ for large $\tau_z$ (Fig. 8D). Jumps in $f$ occur at moderate values of $\tau_z$ at every $\tau_z$ value for which $N_S$ increases by 1.

Comparing experimental results, modeling and theory. We find that both in the experiment and in the simulations: (i) Blocking $g_M$ increases the $N_S$ evoked by brief stimuli (Fig. 3A, two left panels; Fig. 8A) and eventually transfers the neuron to a bistable tonic firing mode (Fig. 3A, third panel from left; Fig. 8A). (ii) Blocking $g_{NaP}$ after the $g_M$ blockage causes the neuron to fire only one spike in response to a brief pulse (Fig. 3A, right panel; Fig. 8A). (iii) In response to a prolonged pulse, blocking $g_M$ can transfer the neuron to a high plateau depolarization state (Fig. 3B, two left columns; Fig. 8B). (iv) If $g_{NaP}$ is also blocked, the neuron fires in a regular mode or becomes quiescent (Fig. 3B, right column; Fig. 8B). The conclusions of the analysis that are demonstrated in the simulations (Fig. 8) are: (i) $g_M$ should be strong enough, but not too strong, to obtain bursting; (ii) $N_S$ decreases with $g_M$; and (iii) $f$ is proportional to $1/\tau_z$.

Note that the firing frequency of the real neuron under the effects of both linopirdine and riluzole is of order 10 Hz (Fig. 3B), that corresponds to the firing pattern denoted by $1_{slow}$ in Fig. 8B, and not to $1_{fast}$. 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 than 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, second column to Fig. 8B1). This is because the model does not include dynamical processes with very slow time scale, such as slow inactivation of $I_{NaP}$ or of $I_{Na}$ (e.g., French et al., 1990; Fleidervish et al. 1996, Mickus et al. 1999).

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

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

*Reducing [Ca\textsuperscript{2+}]\textsubscript{o} is not equivalent to blocking Ca\textsuperscript{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\textsuperscript{2+}-gated currents or Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents, are shown in Fig. 10. Increasing \(g_{\text{Ca}}\) with all Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents blocked (\(g_C = g_{\text{sAHP}} = 0\)) augmented \(N_S\) for both brief and prolonged stimuli (Fig. 10AB) and weakly reduced \(f\) (Fig. 10C). In order to assess the effects of Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductances, we set \(g_{\text{Ca}} = 0.08 \text{ mS/cm}^2\) and computed \(N_S\) as a function of \(\theta_p\) and either \(g_C\) or \(g_{\text{sAHP}}\). As expected, increasing \(g_C\) suppressed burstiness and decreased \(N_S\) for a specific value of \(\theta_p\) (Fig. 10DE); \(f\) increased with \(g_C\) (Fig. 10F). Increasing \(g_{\text{sAHP}}\) 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 \(I_{\text{sAHP}}\) is slow, and the burst is terminated by \(I_M\) before \(I_{\text{sAHP}}\) becomes strong enough to have a considerable effect.

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

The increase in burstiness upon reducing [Ca\textsuperscript{2+}]\textsubscript{o} resulted mainly from the decrease in \(\theta_p\). However, the decrease of \(g_{\text{Ca}}\) per se also could affect \(N_S\). Though in most cases blocking \(g_{\text{Ca}}\) did not
change \( N_5 \) (e.g., Fig. 9D and points 9A,D in Fig. 10H), there were also conditions in which blocking \( g_{Ca} \) transferred the system to a higher level of burstiness (for example, when \( g_{Ca} = 0.2 \text{ mS/cm}^2 \) and \( \theta_p = -45 \text{ mV} \), Fig. 10H). Similarly, though blocking \( g_C \) did not lead to bursting for our reference parameter set (Fig. 9E), it could increase \( N_5 \) for lower values of \( \theta_p \) (Fig. 10DE). 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, 9A-C; Fig. 10G,H) that reducing \([Ca^{2+}]_o\) increases the propensity for bursting in response to brief and prolonged stimuli and augments \( N_5 \). 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 of these neurons (Su et al. 2001). Examples are shown in Figure 9D, in which \( g_{Ca} \) is set to 0 (other parameters, including \( \theta_p \), are as in Figure 9A), and in Figure 9E, where both \( g_C \) and \( g_{sAHP} \) 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 \( I_M \) (\( z \)), provided two conditions are fulfilled. (i) The kinetics of the spike generating currents (i.e., \( I_{Na} \) and \( I_{Kdr} \)) are fast, though not too fast. (ii) 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 \( I_M \) towards more negative potentials
with retigabine (Tatulian et al. 2001, Wickenden et al. 2000), suppresses bursting behavior in both normal and zero \([Ca^{2+}]_o\) (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 (Fig. 7,8). 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, \(I_{sAHP}\) and \(I_M\) play a similar role in the neuronal dynamics, with \(I_{sAHP}\) 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.

(i) *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 \([Ca^{2+}]_o\), \(g_Ca\) and \(g_C\) (compare Fig. 9 with Fig. 1 here and Fig. 2 in Sue et al, 2001).
(ii) How does the propensity for bursting and $N_S$ vary with $I_{\text{app}}, g_{\text{NaP}}$ and $g_M$? In the neuron model (Fig. 7) as well as in native neurons (Fig. 2), $N_S$ increases with $g_{\text{NaP}}$ and depends only weakly on $I_{\text{app}}$. 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).

(iii) How does the propensity for bursting and $N_S$ vary with $g_{\text{Ca}}, g_C$ and $[\text{Ca}^{2+}]_o$? It has been reported that reducing $[\text{Ca}^{2+}]_o$, but not blocking the $\text{Ca}^{2+}$ current or $\text{Ca}^{2+}$-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 $[\text{Ca}^{2+}]_o$ causes $\theta_p$ to shift to more positive voltage. Fig. 10 suggests that in principle, however, there may be cases in which $N_S$ increases with the blockade of $g_C$ (Fig. 10 D,E) or $g_{\text{Ca}}$ (Fig. 10 G,H).

Discrepancies between modeling and experimental results. The dynamical equations of the model do not include slow processes ($> 1 \text{ s}$). 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 column).

In this paper, we show several diagrams in which $N_S$ varies as a function of two parameters (Figs. 7, 8, 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 $g_{\text{NaP}}$ may transfer a high plateau neuron to a neuron spiking at high rates. Fig. 8B does not overlap the example of Fig. 3, in which $g_M$ blockade by linopirdine followed by $g_{\text{NaP}}$ blockade by riluzole transfers a bursting pattern to a high plateau and then to a tonic firing pattern (with firing frequency of about 10 Hz). It is possible, however, that linopirdine and riluzole, used to blocked $g_M$ and $g_{\text{NaP}}$ respectively, exert additional effects on neuronal excitability. Note that Fig. 3A is consistent with Fig. 8A regarding the effects of blocking $g_M$ and $g_{\text{NaP}}$ 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 $g_{\text{NaP}}$
leads to firing of one spike only.

For the model, we chose a $\theta_h$ value that is about 20 mV depolarized compared to available voltage-clamp experimental measurements (Colbert and Pan 2002; Gasparini and Magee 2002; Martina and Jonas 1997), in order 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 $\theta_m-\theta_h$ (e.g., 4.2 mV in (Pinsky and Rinzel 1994; Traub et al. 1991; Traub and Miles 1991)) or by assuming a very large $g_{Na}$ 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 mV 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, 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 in order 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\textsuperscript{2+} and K\textsuperscript{+} (mostly Ca\textsuperscript{2+}-dependent) currents in the apical dendrites (Pinsky and Rinzel, 1994; Traub et al. 1991; Traub and Miles, 1991; Mainen and Sejnowski, 1996; Bazhenov et al. 2004). 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 $g_{NaP}$ and its decrease with $g_{M}$, 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 $g_{NaP}$ ultimately will lead to prolonged bursts with many low-amplitude spikes (Fig. 8) or to high plateau depolarization. Also, increasing $g_{Ca}$ in normal $[Ca^{2+}]_o$ will moderately decrease the propensity for bursting when Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents are operative and strong enough, and will moderately increase this propensity when these currents are blocked (Figs. 9,10). 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 $I_A$ pharmacologically, after which backpropagating spikes evoke dendritic Ca$^{2+}$ spikes which, 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 or less during epileptic seizures (Pumain et al., 1985), are expected to induce "square wave" bursting in CA1 pyramidal cells in vivo. Likewise, factors that upmodulate $g_{NaP}$ (e.g., hypoxia or the accumulation of nitric oxide; Hammarstrom and Gage, 2002), or downmodulate $g_M$ (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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FIGURE LEGENDS

Figure 1. Effects of lowering [Ca$^{2+}$]$_o$ on the firing mode of a truncated CA1 pyramidal cell. A, in normal ACSF (2 mM Ca$^{2+}$) the neuron fired a solitary spike in response to suprathreshold brief depolarizing current pulses (leftmost panel). Sequentially lowering [Ca$^{2+}$]$_o$ to 1.2 mM (middle panel) and to nominally 0 mM (rightmost panel) caused spike ADP augmentation which converted the single spike response to a burst of 2 and 3 spikes, respectively. B, stimulating with longer (180 ms) depolarizing current pulses of increasing intensity induced repetitive firing. In normal ACSF the neuron generated an accommodating train of independent spikes (leftmost panel). Reducing [Ca$^{2+}$]$_o$ to 1.2 and to nominally 0 mM converted these responses to repetitive bursting with much less adaptation (middle and rightmost panels).

Figure 2. The $I_{NaP}$ blocker riluzole suppresses bursting in nominally 0 [Ca$^{2+}$]$_o$. A, exposing this truncated neuron to nominally 0 Ca$^{2+}$ ACSF converted it to a low-threshold bursting mode (leftmost column). Adding 10 µM riluzole to the ACSF suppressed bursting within 24 min of exposure (rightmost column). B, in the same neuron, repetitive bursting evoked by long depolarizing current pulses (leftmost column) was converted to a regular firing mode by riluzole (rightmost column).

Figure 3. The $I_M$ blocker linopirdine converts repetitive bursting in nominally 0 [Ca$^{2+}$]$_o$ to plateau depolarizing potentials. A, exposing this truncated neuron to nominally 0 Ca$^{2+}$ ACSF converted it to a low-threshold bursting mode (leftmost column). Exposing the neuron to 10 µM linopirdine for 20 min converted the 3-spikes burst to a prolonged burst followed by a sustained depolarizing lasting hundreds of milliseconds (middle column and inset). The burst and associated plateau depolarization were readily suppressed by subsequent addition of 10 µM riluzole (rightmost panel). B, in the same neuron, repetitive bursting evoked by long depolarizing current pulses (leftmost column) was converted to a sustained plateau potential (middle column), which was converted to a regular firing by riluzole (rightmost column).

Figure 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 \( I_{\text{app}}=0 \mu \text{A/cm}^2 \) in the neuron model at \([\text{Ca}^{2+}]_o=0\). Other parameters are: \( g_{\text{NaP}}=0.3 \text{ mS/cm}^2 \), \( V_L=-62 \text{ mV} \).

**Figure 5.** Theory: fast-slow analysis of the neuronal firing patterns. Bifurcation diagrams of the fast subsystems are plotted with \( z \) considered as a parameter for \( g_{\text{NaP}}= 0 \) (A), \( g_{\text{NaP}}=0.2 \text{ mS/cm}^2 \) (B), \( g_{\text{NaP}}=0.3 \text{ mS/cm}^2 \) (C), and \( g_{\text{NaP}}=0.41 \text{ mS/cm}^2 \) (D); \( I_{\text{app}}=1 \mu \text{A/cm}^2 \). Solid lines denote stable states and dotted lines denote unstable states. Thin black lines denote rest state, and thick black lines denote the minimal and maximal voltages of periodic, tonic firing states (limit cycles). The green curve denotes the curve \( z=z_T(V) \) (Table 1). The blue curve denotes the value of \( V_{\text{equiv}} \) (Eq. 5) during periodic firing. Violet solid circles denote Hopf bifurcations. The voltage time course of the neuron in the full system (including \( z \) as a variable) is denoted by the red curve. Red arrow denotes the direction of that curve in the \( z-V \) plane.

**Figure 6.** Modeling: variant firing patterns of the neuron model for \([\text{Ca}^{2+}]_o=0\) and various values of \( g_{\text{NaP}} \). Parts \( A-D \) correspond to the following values of \( g_{\text{NaP}} \) (in \( \text{mS/cm}^2 \)): \( 0 \) (A), \( 0.08 \) (B), \( 0.18 \) (C), \( 0.3 \) (D). The neuron was at rest without current injection (\( V_L \) is set to -70 mV and therefore \( V \) is about -72 mV). In each part, panels (a) and (b) show responses of the neuron model to strong \( (I_{\text{app,th}}+0.3\mu \text{A/cm}^2; \ a) \) and weak \( (I_{\text{app,th}}+0.05\mu \text{A/cm}^2; \ b) \) prolonged stimuli, respectively; \( I_{\text{app,th}} \) is the minimal \( I_{\text{app}} \) required to attain spike threshold. The values of \( I_{\text{app,th}} \) (in \( \mu \text{A/cm}^2 \)) are 0.84, 0.59, 0.46 and 0.36 for \( g_{\text{NaP}} \) (in \( \text{mS/cm}^2 \)) values of 0, 0.08, 0.18, 0.3 respectively. Panel (c) presents the membrane potential in response to a brief (3 ms) current pulse with a density of \( I_{\text{app,th}}+2.5\mu \text{A/cm}^2 \). The values of \( I_{\text{app,th}} \) (in \( \mu \text{A/cm}^2 \)) are 7.1, 6.0, 5.3 and 4.7 for \( g_{\text{NaP}} \) (in \( \text{mS/cm}^2 \)) values of 0, 0.08, 0.18, 0.3 respectively. The horizontal dashed line represents -80 mV. The injected current pulses are indicated below the voltage traces. These currents are always 0 at the starting time. It is evident that increasing \( g_{\text{NaP}} \) enhances burstiness in the neuron model.

**Figure 7.** Modeling: \( A,B \), dependence of \( N_S \), the number of intraburst spikes, on \( g_{\text{NaP}} \) and \( I_{\text{app}} \) for \([\text{Ca}^{2+}]_o=0\). The number in the bounded regions ("2", "1", etc.) denote \( N_S \); "0" denotes no firing. The
solid circles denoted by letters, such as "Da", indicate the corresponding panel in Fig. 6. A, Values of \( N_S \) in bursts evoked by brief stimuli. There were two discontinuous regimes in which 2 spikes were obtained at low and at high values of \( I_{\text{app}} \), whereas only one spike was elicited at intermediate \( I_{\text{app}} \) regions. The solid circle on the top-right region of map (A) denotes the parameter set of a burst of fast low-amplitude spikes, plotted right to the map. The time course of the applied current is plotted below the voltage time course. B, Values of \( N_S \) in bursts evoked by prolonged stimuli. For a given \( g_{\text{NaP}} \), prolonged pulses evoked more intraburst spikes than brief pulses (at least when \( I_{\text{app}} \) was not extremely large). The dotted lines correspond to the \( I_{\text{app}} \) and \( g_{\text{NaP}} \) values for panels C and D respectively. C, dependence of the bursting frequency \( f \) on \( g_{\text{NaP}} \) for \( I_{\text{app}}=1 \) \( \mu \)A/cm\(^2\). D, dependence of the \( f \) on \( I_{\text{app}} \) for \( g_{\text{NaP}} =0.3 \) mS/cm\(^2\).

Figure 8. Modeling. A,B, dependence of \( N_S \) on \( g_{\text{NaP}} \) and \( g_{\text{M}} \) for [Ca\(^{2+}\)]\(_o\)=0; \( \tau_z=75 \) ms. Symbols are as in figure 7. A, Values of \( N_S \) in bursts evoked by brief stimuli with \( I_{\text{app}}=7 \) \( \mu \)A/cm\(^2\). Interestingly, \( N_S \) could switch abruptly from 1 to a value larger than 2 as \( g_{\text{NaP}} \) increased. For example, for \( g_{\text{M}}=0.8 \) mS/cm\(^2\) , \( N_S \) switched from 1 to 3 at \( g_{\text{NaP}}=0.23 \) mS/cm\(^2\). B, Values of \( N_S \) in bursts evoked by prolonged stimuli with \( I_{\text{app}}=1 \) \( \mu \)A/cm\(^2\). The symbols "1fast" and "1slow" denote tonic firing with fast (inter-spike interval of order 10 ms) and slow (inter-spike interval of order 100 ms), respectively. The transition from the parameter regime at which \( N_S=2 \) (left "finger", \( g_{\text{M}}=0.6 \) mS/cm\(^2\)) to the regime of normal bursting was very complex and involved chaotic dynamics. The dotted line denotes the \( g_{\text{NaP}} \) value used for panel C. The solid circles denoted by I-III in each map represent the parameter sets of the firing patterns shown right to the maps. The time course of the applied current is plotted below the voltage time courses. C, dependence of the bursting frequency \( f \) on \( g_{\text{M}} \). Parameters: \( I_{\text{app}}=1 \) \( \mu \)A/cm\(^2\), \( g_{\text{NaP}} =0.25 \) mS/cm\(^2\), \( \tau_z=75 \) ms. D, dependence of the bursting frequency \( f \) on \( 1/\tau_z \). Parameters: \( I_{\text{app}}=1 \) \( \mu \)A/cm\(^2\), \( g_{\text{NaP}} =0.25 \) mS/cm\(^2\), \( g_{\text{M}} =1 \) mS/cm\(^2\).

Figure 9. Modeling: variant firing patterns of the neuron model for different values of [Ca\(^{2+}\)]\(_o\). The sets of parameters \{\( g_{Ca} \) (mS/cm\(^2\)), \( g_{C} \) (mS/cm\(^2\)), \( g_{SAHP} \) (mS/cm\(^2\)), \( \theta_0 \) (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 $[\text{Ca}^{2+}]_o$ 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, that eliminates Ca$^{2+}$-dependent K$^+$ currents. The neuron model is at rest before the current injection. Each column in the figure includes three voltage traces. The panels in (a) and (b) show the responses of the neuron model to prolonged strong and weak stimuli, respectively. Panels (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 panels (a), 0.7 $\mu$A/cm$^2$ for panels (b) and 7 $\mu$A/cm$^2$ for panels (c). Reducing $[\text{Ca}^{2+}]_o$ (A-C), but neither blocking $g_{\text{Ca}}$ (D) nor blocking $g_{C}$ and $g_{\text{sAHP}}$ (E), induces bursting in a regular firing cell.

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

- **A.** 0.0
- **B.** 0.08
- **C.** 0.18
- **D.** 0.3

- **-80 mV**
- **b**
- **I 1 \mu A/cm^2**
- **200 ms**
- **20 mV**
- **10 \mu A/cm^2**
- **20 ms**
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<th>Ref./ Com.</th>
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<td>$dh/dt = \phi[h_v(V) - h]/\tau_h(V)$</td>
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<td>$I_{M, z}$</td>
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<td>$\theta_z=-39 \text{ mV, } \sigma_z=5 \text{ mV.}$</td>
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Table 1: Kinetics equations and parameters for the zero [$Ca^{2+}$]_o model.

The activation and inactivation curves $x_v(V)$ are determined by the equation $x_v(V)=\{1+\exp[-(V-\theta_x)/\sigma_x]\}^{-1}$ where $x=m,h,n,a,b,z$. References and comments: 1. Fleidervish et al. 1996; Martina and Jonas 1997; Sah et al. 1988b; Golomb and Amitai 1997. The difference between $\theta_m$ and $\theta_h$ is smaller than what is usually measured in pyramidal cell somata, partially because the activation curve of axons in pyramidal cells is shifted towards 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 $I_{NaP}$ to be instantaneous. Recent experiments in CA1 pyramidal cells show that reducing Ca$^{2+}$ concentration leads to a significant negative shift of $\theta_p$ (Yue et al. 2005; see also Li and Hatton 1996). Therefore, $\theta_p$ is varied throughout the paper (typically between $-47 \text{ mV}$ at 0 [$Ca^{2+}$]_o and $-41 \text{ mV}$ at physiological [$Ca^{2+}$]_o values). 3. Martina et al. 1998; Sah et al. 1988a. 4. Hoffman et al. 1997; Rush and Rinzel, 1995. 5. Halliwell and Adams, 1982.
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| $I_{Ca, r}$      | $dr/dt = \left[ r_s(V) - r \right] / \tau_r$  
$r_s(V) = \left[ 1 + \exp\left( - \left( V - \theta_r \right) / \sigma_r \right) \right]^{-1}$ | $\tau_r=1$ | $\theta_r=-20$ mV, $\sigma_r=10$ mV | 1 |
| $I_{C, c, d}$    | $dc/dt = \left[ c_s(V) - c \right] / \tau_c$  
d = $d_s([Ca]_i)$  
c_s(V) = $\left[ 1 + \exp\left( - \left( V - \theta_c \right) / \sigma_c \right) \right]^{-1}$  
d_s([Ca^{2+}]) = $\left( 1 + a_c / [Ca^{2+}] \right)^{-1}$ | $\tau_c=2$ | $\theta_c=-30$ mV, $\sigma_c=7$ mV, $a_c=6$ | 2 |
| $I_{sAHP, q}$    | $dq/dt = \left[ q_s([Ca]_i) - q \right] / \tau_q$  
$q_s([Ca]_i) = \left( 1 + a_q^4 / [Ca^{2+}] \right)^{-1}$ | $\tau_q=450$ | $a_q=2$ | 2 |

Table 2: Kinetics equations and parameters for the non-zero [Ca^{2+}]_o model.

References and comments: 1. Kay and Wong 1987. 2. Lancaster and Adams 1986. The current $I_C$ is fast and is controlled by both $V$ and $[Ca^{2+}]$, whereas $I_{sAHP}$ is slow and is controlled by $[Ca^{2+}]$ only.