Characterization of Voltage-Gated K\textsuperscript{+} Currents Contributing to Subthreshold Membrane Potential Oscillations in Hippocampal CA1 Interneurons

France Morin,1,* Darrell Hauffer,2,3,* Frances K. Skinner,2,3,4 and Jean-Claude Lacaille1

1Le Groupe de Recherche sur le Système Nerveux Central, Département de Physiologie, Université de Montréal, Montreal, Quebec; 2Toronto Western Research Institute, University Health Network; and 3Departments of Physiology and 4Medicine (Neurology), Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada

Submitted 17 September 2009; accepted in final form 11 April 2010


INTRODUCTION

In behaving animals, patterns of hippocampal synchronous oscillatory activity in the theta-frequency range are linked to the total K\textsuperscript{+} current was \( I_{K_{fast}} > I_{K_{slow}} > I_{NaP} \). The presence of \( I_{K_{fast}} \) and the relative contributions of K\textsuperscript{+} currents in LM/RAD-INs are different from those of other CA1 interneurons, suggesting the presence of differential complement of K\textsuperscript{+} currents in subgroups of interneurons. We next determined whether these K\textsuperscript{+} currents were sufficient for MPO generation using a single-compartment model of LM/RAD-INs. The model captured the subthreshold voltage dependence of MPOs. Moreover, all K\textsuperscript{+} currents were active at subthreshold potentials but \( I_{Na} \), \( I_{K_{fast}} \), and the persistent sodium current \( I_{NaP} \) were most active near threshold. Using impedance analysis, we found that \( I_{K_{fast}} \) and \( I_{NaP} \) contribute to MPO generation by modulating peak spectral frequency during MPOs and governing the voltage range over which MPOs occur. Our findings uncover a differential expression of a complement of K\textsuperscript{+} channels that underlies intrinsic rhythmic activity in inhibitory interneurons.

METHODS

Slice preparation

All animal procedures conformed to the animal welfare guidelines of the Université de Montréal (CDEA, Université de Montréal, Quebec; Centre-Ville, Montréal, QC, Canada H3C 3J7 (E-mail: jean-claude.lacaille @umontreal.ca)).

Address for reprint requests and other correspondence: J-C. Lacaille, Université de Montréal, Département de Physiologie, CP 6128, succ. Centre-Ville, Montréal, QC, Canada H3C 3J7 (E-mail: jean-claude.lacaille @umontreal.ca).

These authors contributed equally to this work.

* These authors contributed equally to this work.
be, Canada). Sixteen- to 20-day-old male rats (Sprague–Dawley, n = 46; Charles River Laboratories, Senneville, Quebec, Canada) were deeply anesthetized with halothane (MTC Pharmaceuticals, Cambridge, Ontario, Canada). The brain was dissected in ice-cold (0–4°C) artificial cerebrospinal fluid (ACSF) and transverse hippocampal slices (300 μm thick) were cut using a vibratome (Model VT1000S; Leica, Wetzlar, Germany). After approximately 1 h, hippocampal slices were transferred to the recording chamber and superfused with oxygenated ACSF (2 ml/min) at room temperature (20–22°C). Slices were viewed with an upright microscope (Zeiss Axioskop; Carl Zeiss, Oberkochen, Germany) equipped with Hoffman optics (Modulation Optics, Greenvale, NY), a long-range water immersion objective (×40), and an infrared video camera (Model 6500; Cohu Electronics, San Diego, CA). Recordings were made with an Axopatch 200B or a multiclamp 700B amplifier (Molecular Devices, Foster City, CA) and signals were filtered at 2 kHz (eight-pole Bessel filter) and digitized at 10 kHz on a Pentium based computer using pClamp 9.0 (Molecular Devices).

Electrophysiological recordings
Voltage-clamp recordings of K⁺ currents were made in ACSF containing (in mM): 125 NaCl, 25 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 2 MgCl₂, and 23 glucose, saturated with 95% O₂-5% CO₂ (pH 7.4, 310 mOsm). Patch pipettes pulled from borosilicate glass tubing (1.2 mm OD, 2–3 MΩ; WPI) were filled with (in mM): 120 K-gluconate, 20 KCl, 10 HEPES, 5 EGTA, 1 MgCl₂, 5 glutathione, 2 ATP-Tris, 0.4 GTP-Tris, and 0.05% Oregon Green dextran (Invitrogen Canada, Burlington, Ontario, Canada) (pH adjusted between 7.2 and 7.3 with KOH, 295 mOsm). Outside-out patches were isolated from somata of CA1 interneurons located at the border of LM/RAD-INTs. Generally, membrane patches excised from somata in our outside-out patch recordings had membrane areas similar to those of nucleated patches (as illustrated in Fig. 1A and in Fig. 1Ac in Lien et al. 2002). The inclusion of the fluorescent dye Oregon Green in the recording solution was used to confirm that we did not record from pyramidal cells. Because of the use of the outside-out patch configuration, cell labeling was successful in roughly 30% of recordings and in all cases these were interneurons. Interneurons had mean resting potential of −68.8 ± 0.7 mV and were maintained at −73 mV during voltage-clamp experiments. The injected current to maintain outside-out recordings at −73 mV was continuously monitored and only recordings with stable holding current were included in the analysis. Leakage and capacitative currents were subtracted on-line using a P/4 procedure (Lien et al. 2002; Zhang and McBain 1995). Series resistance was compensated by 60%, except for recordings of small amplitude currents for which it was not compensated. Traces shown are either single traces (activation and inactivation protocols) or the average of five sweeps given at 0.125 Hz intervals. Potassium current subtypes were characterized in the presence of tetrodotoxin (TTX, 0.5 μM) to block Na⁺ currents and low or high concentrations of TEA (0.5 and 20 mM) to prevent fast or slow delayed rectifier K⁺ currents (Lien et al. 2002), respectively. The Kv1.1, Kv1.2, and Kv1.6 potassium channel blockers α-dendrotoxin (α-DTX, 500 nM) and low doses of 4-aminopyridine (4-AP, 60 μM) were bath-applied in some experiments. When α-DTX was used, bovine serum albumin (0.1%, Jackson ImmunoResearch Laboratories, Toronto, Canada) was added to the extracellular solution.

Whole cell current-clamp recordings of membrane potential oscillations (MPOs) were made in ACSF containing (in mM): 124 NaCl, 26 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 2 MgSO₄, and 10 glucose, saturated with 95% O₂-5% CO₂ (pH 7.4, 300 mOsm). Recording pipettes (1.0 mm OD, 3–6 MΩ) were filled with (in mM): 140 K-glucuronate, 5 NaCl, 10 HEPES, 0.5 EGTA, 2 MgCl₂, 2 ATP-Tris, 0.4 GTP-Tris, and 0.1% biocytin (285 mOsm). Biocytin-filled interneurons were recovered in 83% of cases and morphology confirmed that recorded cells were interneurons. Interneurons had mean resting potential of −70.8 ± 1.2 mV and series resistance was compensated using the bridge balance technique, ranging between 9 and 24 mΩ (n = 12). The liquid junction potentials for voltage- and current-clamp experiments were 13 and 14 mV, respectively, and were corrected.

All chemicals were purchased from Sigma except for tetrodotoxin and α-DTX, which were purchased from Alomone Labs (Jerusalem, Israel).

Data analysis
Peak current amplitudes (peak component for Iₖ, Iₖfast, and IₖD and plateau component for Iₖslow) were measured from baseline. To obtain the activation curve of K⁺ currents, we calculated the chord conductance (g) by dividing respective peak currents at different test potentials by the driving force, assuming ohmic behavior and a reversal potential of −101 mV (measured using reversal of tail currents; data not shown). The activation curves of K⁺ currents were obtained by fitting the curve with a Boltzmann function of the form

\[ g/g_{\text{max}} = \frac{1}{1 + \exp[-(V - V_{1/2})/k]} \]

where \( g/g_{\text{max}} \) is the conductance normalized to its maximal value, \( V \) is the membrane potential, \( V_{1/2} \) is the membrane voltage at which the current amplitude is half-maximum, and \( k \) is the slope factor. The inactivation curve of A-type K⁺ currents was fitted with the Boltzmann function

\[ I/I_{\text{max}} = \frac{1 + \exp[(V - V_{1/2})/k]}{1} \]

where \( I/I_{\text{max}} \) is the current normalized to its maximal value. To account for the remaining sustained K⁺ current in the presence of 20 mM TEA, we subtracted K⁺ currents that remained with a prepulse of 30 mV from K⁺ currents evoked at all potentials.

The inactivation curves of Iₖfast and Iₖslow were best fitted with a Boltzmann function plus a constant

\[ I/I_{\text{max}} = A[1 + \exp(-(V - V_{1/2})/k)]^{-1} + (1 - A) \]

where \( A \) is the fraction of channels that inactivate.

Segments of membrane potential recordings used for spectral analysis of MPOs were low-pass filtered at 100 Hz and the sampling rate was reduced to 1 kHz. The average power spectra were calculated from three 2.048-s-duration segments without action potentials (Chapman and Lacaille 1999a) using Clampfit 9.0 (Molecular Devices). Changes in peak frequency (measured between 1.5 and 5 Hz) and total power were assessed following drug applications using Student’s paired t-test (\( P < 0.05 \)). Data are reported as means ± SE.

Single-compartment interneuron model
The model is given by the current balance equation

\[ C \frac{dV}{dt} = I_{\text{applied}} - (I_{\text{NaT}} + I_{\text{Nap}} + I_{\text{Kfast}} + I_{\text{Kslow}} + I_A + I_D + I_{\text{leak}}) \]

where \( C \) is the specific membrane capacitance (in \( \mu \text{F/cm}^2 \)), \( V \) is the membrane potential (in mV), and the \( I \) are the membrane currents. The transient and persistent sodium currents (\( I_{\text{NaT}} \) and \( I_{\text{Nap}} \), respectively), the fast and slow delayed rectifiers (\( I_{\text{Kfast}} \) and \( I_{\text{Kslow}} \), respectively), and α-DTX-sensitive current (\( I_D \)) were all modeled according to the Hodgkin–Huxley (HH) formalism. \( I_A \) represents an A-type K⁺ current mediated by Kv4.3 (Bourdeau et al. 2007). Detailed multistate models exist for Kv4.3 (Wang et al. 2004, 2005) and we used a simplified version of these models (see details in the APPENDIX). \( I_{\text{leak}} \) is the passive leak conductance current with conductance set to match the membrane time constant for LM/RAD-INTs given in Chapman and Lacaille (1999a). Further model details and parameter values are provided in the APPENDIX.
The applied current \( I_{\text{applied}} \) consists of a mean offset (DC) component and a white noise component

\[
I_{\text{applied}} = I_{\text{DC}} + I_{\text{Noise}}
\]

Implementation of the noise term is described in Numerics. The intensity of noise was chosen to generate MPOs of about 1 mV in amplitude immediately subthreshold to action potential generation.

The DC component was used as a control parameter to depolarize the model cell and represents injected current to the LM/RAD-IN under current clamp. Although the noise is independent of channel activity in the model, our interpretation is that the noise is intrinsically generated (e.g., through the stochastic gating of ion channels). This is consistent with the intrinsic presence of MPOs in the absence of synaptic input as observed experimentally.
The maximal conductance values of the potassium currents were set based on the maximum and relative current amplitudes from experimental LM/RAD-IN data. To compute K⁺ current amplitudes, we used the mean patch capacitance of 0.57 pF and assume specific membrane capacitance of 1 μF/cm². The mean peak current for $I_A$ patch recordings, based on a voltage step to 57 mV, was 660.2 pA. An estimate for surface area of clamped membrane is

$$\frac{0.57 \times 10^{-12} F}{1 \times 10^{-6} F/cm^2} = 0.57 \times 10^{-6} \text{ cm}^2$$

and current density for $I_A$ is

$$\frac{660 \text{ pA}}{0.57 \times 10^{-6} \text{ cm}^2} = 1,158.2 \mu A/cm^2$$

From simulation, the channel open probability (gating variable) at the peak $I_A$ current (maximal outward current) at 57 mV was 0.376. The maximal conductance required for a peak outward current of 1,158.2 μA/cm² is thus

$$s_{\text{max}} = \frac{-1,158.2 \mu A/cm^2}{(0.376)(-101 \text{ mV} - 57 \text{ mV})} = 19.5 \text{ mS/cm}^2$$

Analogous computations were performed for the other potassium currents with maximal conductance values given in Appendix Table A1.

**Numerics**

Model and voltage-clamp simulations were performed in XPPAUT (Ermentrout 2002). Numerical integration was done using the forward Euler method, with a step size of 0.05 ms. MATLAB was used for analyses and to automate calls to XPPAUT and change parameter values using the freely available XPP-MATLAB interface written by Rob Clewley (http://www.math.pitt.edu/~bard/xpp/xpp.html). Multiple runs were performed simultaneously using a Linux cluster available through Research Information Systems at the University Health Network. The cluster was composed of 42 nodes, with each node consisting of a dual 3.0 GHz Xeon processor with 2 Gb of RAM. All other computing was performed on a 3.2 GHz Pentium 4 PC running Linux. The noise term for model simulations was simulated in XPPAUT using Wiener parameters (Ermentrout 2002), scaled by an intensity of 0.05. We note that the exact noise intensity used is not critical, so long as it is sufficiently small for the linear approximations made in the model analysis to hold (described in the following text).

**Model analysis**

We used impedance analysis to examine our single-compartment model. In impedance (or resonance) analysis, typically one injects sinusoidal current (such as a ZAP function) into the cell, records the voltage, and calculates the impedance function from which one can determine whether there is an impedance peak, a resonance, at particular frequencies. The effect of different currents can be examined by blocking particular currents and determining how resonance is affected (e.g., Hu et al. 2002). Here, because we have a mathematical model of the system, we can deal directly with the currents, thus providing not only insight into the effect of the different currents, but also computational efficiency. We perform computational resonance analyses on the constituent currents and these responses can then be manipulated to obtain the voltage response of the system. This is done as follows.

**Constructing the Response Function.** The solution of the current balance equation

$$\frac{dV}{dt} = \frac{1}{C_m} (I_{\text{DC}} + I_{\text{Noise}} - \sum I_k) \quad (I)$$

can be expressed around the subthreshold steady-state solution, provided that certain restrictions on the behavior of the currents are met (outlined in the following text). Our aim was to express the output of the system at a given frequency ω and level of depolarization $V_{ss}$ as a product of the noise component at ω and a response function $H(\omega, V_{ss})$, which is computed over a range of ω and $V_{ss}$. The fixed point of the system is determined by $I_{\text{DC}}$, which parametrizes the steady-state values for the membrane potential ($V_{ss}$) and currents ($I_{k,ss}$), although the parameterization is not explicitly shown. We assumed that all oscillatory activity in the currents and membrane potential is centered at these fixed values. This is reasonable if the scale of oscillations is small with respect to the nonlinearity in the current activation properties. We further assumed that for sinusoidal membrane potential drive at frequency $\omega$, the current response is a pure sinusoid at ω (no harmonics are present) and that the response scales linearly with the amplitude of the drive. We now proceed to construct $H(\omega, V_{ss})$.

We fix the membrane potential to be a sinusoid with amplitude $V_0$ centered at $V_{ss}$ and we represent the oscillatory component using complex exponential notation

$$\hat{V} = V_0 e^{i\omega}$$

This is related to the observed signal through

$$V(t) - V_{ss} = V_0 \cos (2\pi \omega t + \varphi) = \text{Re} \{e^{i2\pi \omega t} \hat{V}\}$$

We apply this fixed voltage signal to each current model and compute the steady-state current response (allowing all transients to decay to zero). If the oscillatory voltage is of sufficiently small amplitude, the currents can reasonably be approximated by a constant plus the sinusoidal drive scaled by $A_k$ and phase shifted by $\varphi_k$

$$I_k = I_{k,ss} + A_k e^{i\varphi_k} \quad (2)$$

Note that $A_k$ and $\varphi_k$ are functions of the driving frequency $\omega$ and the depolarization level $V_{ss}$. We also define

$$A_k e^{i\varphi_k} = \sum_k A_k e^{i\varphi_k} \quad (3)$$

so that

$$\sum_k I_k = \sum_k I_{k,ss} + A e^{i\varphi} \hat{V} \quad (4)$$

Because we are examining the system near steady state, we expect that

$$\sum_k I_{k,ss} = I_{\text{DC}} \quad (5)$$

Substituting Eqs. 4 and 5 into Eq. 1 and expressing the time-dependent voltage derivative in complex notation gives

$$2\pi i \omega \hat{V} = \frac{1}{C_m} (i \hat{V}_{\text{Noise}} - A e^{i\varphi} \hat{V})$$

which can be rearranged to give

$$\hat{V} = \left[ -\frac{1}{2\pi i \omega C_m + A e^{i\varphi}} \right] \hat{V}_{\text{Noise}} \quad (6)$$

The term in brackets in Eq. 6 is our system response function $H(\omega, V_{ss})$ and this is what is plotted in Figs. 8B and 9 and Supplemental Fig. S2. $\hat{V}_{\text{Noise}}$ denotes the component of the noise signal at frequency ω.

**Results**

**Pharmacological characterization of K⁺ current subtypes**

To investigate the complement of K⁺ channels that are expressed in LM/RAD-INs, we recorded from outside-out patches of interneuron somata and used pharmacological blockers to isolate K⁺ current subtypes (Fig. 1). The use of TEA to differentiate distinct components of K⁺ currents is not
entirely selective for specific channels. For example, low doses of TEA may block Kv3, BK, and some Kv1 channels, whereas Kv2 and Kv7 channels, whereas Kv2 and Kv7 currents, the half-maximal inhibitory concentrations (IC50 values) were 17 ± 7 μM and 8.9 ± 6.4 mM (Fig. 1A), with blocked fractions of 65 and 35%, respectively. Similarly measured for the plateau component of K+ currents (Fig. 1C), the IC50 values were 19 ± 19 μM and 8.8 ± 14.0 mM with blocked fractions of 62 and 38%, respectively. Despite application of a high concentration of TEA (20 mM), a portion of the plateau component of the delayed rectifier K+ currents still remained unblocked (Coetzee et al. 1999; Storm 1988). These results suggest that LM/RAD-INs possess at least two K+ current subtypes that are preferentially blocked by low and high concentrations of TEA.

Based on digital subtraction of K+ currents under different pharmacological conditions (Lien et al. 2002) and current kinetics, we distinguished four distinct subtypes of K+ currents in LM/RAD-INs (Fig. 1, D and E). First, we identified a fast delayed rectifier current, sensitive to low TEA (0.5 mM) (IKfast = IC(TTX) − IC0.5 mM TEA), that activated rapidly but that only partially inactivated (Fig. 1D). Second, we isolated a slow delayed rectifier current, partially blocked by a high concentration of TEA (20 mM) (IKslow = IC0.5 mM TEA − IC20 mM TEA), that activated very slowly and that only slightly inactivated. Third, prominent A-type K+ currents remained in high concentrations of TEA that activated and inactivated rapidly. Mean amplitudes of IKfast, IKslow, and A-type K+ currents, evoked with a test pulse to 57 mV, were 257.8 ± 35.1 pA (n = 12), 147.0 ± 40.7 pA (n = 13), and 660.2 ± 201.9 pA (n = 13), respectively (Table 1). Finally, all cells displayed a residual plateau current that remained in the presence of TTX and 20 mM TEA in addition to IA (e.g., Fig. 1D). Thus we examined whether this K+ current component was sensitive to the selective Kv1.1, Kv1.2, and Kv1.6 channel blocker α-DTX (500 nM), which is usually referred to as ID in other cell types (Bekkers and Delaney 2001; Locke and Nerbonne 1997). In this case, K+ currents in the presence of α-DTX were subtracted from currents in TTX and we then identified an ID-like current (ID = IC(TTX) − ICα-DTX) that activated fairly rapidly but had a slow inactivation (Fig. 1E).

The mean amplitude of ID, evoked with a test pulse to 57 mV, was 170.7 ± 33.4 pA (n = 12, Table 1). Not all LM/RAD-INs displayed this K+ current and ID was observed in 12/16 cells. Additional α-DTX experiments were performed in the presence of TTX and high doses of TEA (20 mM), which revealed ID currents with characteristics similar to those of ID isolated in TTX only: mean amplitude (190.5 ± 109.1 μV, n = 5), risetime (2.5 ± 1.3 ms), and decay τ (62.6 ± 16.7 ms). Thus the DTX-sensitive component appears insensitive to TEA.

The relative contribution of each current to the total K+ current was estimated by measuring the individual peak amplitudes of each respective current relative to the total peak amplitude of K+ currents. Because there is a temporal aspect of the relative contribution of each current, the peak amplitudes of IA, IKfast, and ID were measured at their maximal peaks, whereas the peak amplitude of IKslow was measured at the plateau component. As a result, the relative contributions of IA > IKfast > IKslow = ID had values of 49 ± 15, 23 ± 3, 14 ± 4, and 14 ± 4%, respectively (Table 1). These results indicate that LM/RAD-INs express four pharmacologically distinguishable K+ currents activated near threshold. The presence of ID and the relative contributions of these currents differ from those reported in OA-INs (Lien et al. 2002), suggesting that LM/RAD-INs express a differential complement of K+ currents.

Gating properties of K+ current subtypes

**Fast delayed rectifier K+ current**

To understand how these different K+ currents may regulate LM/RAD-INs excitability, we next characterized the gating properties of each K+ current subtype isolated pharmacologically. First, the fast delayed rectifier current was isolated by subtraction of K+ currents in the presence of low concentrations of TEA from K+ currents in the presence of TTX (IKfast = IC(TTX) − IC0.5 mM TEA). IKfast had an

<table>
<thead>
<tr>
<th>Property</th>
<th>IKfast</th>
<th>IKslow</th>
<th>IA</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean amplitude, pA‡</td>
<td>257.8 ± 35.1 (12)</td>
<td>147.0 ± 40.7 (13)</td>
<td>660.2 ± 201.9 (13)</td>
<td>170.7 ± 33.4 (12)</td>
</tr>
<tr>
<td>Activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1/2, mV</td>
<td>−14.3 ± 0.2</td>
<td>−6.4 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k constant, mV</td>
<td>10.7 ± 0.2 (14)</td>
<td>24.5 ± 0.6 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1/2, mV</td>
<td>−6.4 ± 0.7</td>
<td>−60.8 ± 0.8</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>k constant, mV</td>
<td>24.5 ± 0.6 (7)</td>
<td>26.6 ± 0.7 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risetime, ms‡</td>
<td>10.3 ± 4.7 (11)</td>
<td>20.8 ± 5.9 (11)</td>
<td>6.0 ± 0.04 (14)</td>
<td>4.4 ± 1.9 (9)</td>
</tr>
<tr>
<td>Decay, ms‡</td>
<td>67.3 ± 146.4 (4)</td>
<td>301.6 ± 109.0 (6)</td>
<td>11.1 ± 1.6 (13)</td>
<td>49.3 ± 5.0 (9)</td>
</tr>
<tr>
<td>Recovery from inactivation, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>τfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>τslow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deactivation, ms‡</td>
<td>10.1 ± 2.2 (9)</td>
<td>20.4 ± 5.4 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative contribution, %</td>
<td>23 ± 3 (12)</td>
<td>14 ± 4 (12)</td>
<td>49 ± 15 (12)</td>
<td>14 ± 4 (12)</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of patches (n, in parentheses). ‡Measured at 57 mV. †Measured at −43 mV.
activation curve with a midpoint potential of $-14.3 \pm 0.2$ mV and a steep slope factor ($k$ constant) of $10.7 \pm 0.2$ mV (Fig. 2C, Table 1, $n = 14$). The inactivation curve of $I_{Kfast}$ obtained using 1-s prepulses between $-143$ and $27$ mV had a midpoint potential of $-64.6 \pm 0.7$ mV and a slope factor of $24.5 \pm 0.6$ mV ($n = 7$, Fig. 2C, Table 1), with roughly 20% of $I_{Kfast}$ that did not fully inactivate. The percentage of current remaining reflects a constant that is added to the inactivation equation (see METHODS) to obtain an appropriate fit of the inactivation curve. The 20–80% risetime of $I_{Kfast}$, measured with test pulse potentials between $57$ and $-23$ mV, was independent of voltage and ranged between $10.1 \pm 4.1$ and $20.2 \pm 6.2$ ms ($n = 11$, Fig. 2D). The decay time constant of $I_{Kfast}$ was obtained by fitting the subtracted $K^+$ current and thus represents a fast component of a multieponential process because of the presence of a noninactivating component. The decay time constant of $I_{Kfast}$ evoked at $57$ mV was best fitted with a monoeXponential function and was $67.3 \pm 14.6$ ms ($n = 4$, Table 1). The decay time constant was independent of voltage (Fig. 2D). The decay time constant of $I_{Kfast}$ obtained by measuring the decay of tail currents evoked by a pulse to $-43$ mV, was $10.1 \pm 2.2$ ms (Fig. 2E, Table 1, $n = 9$).

**Slow delayed rectifier $K^+$ current.** The slow delayed rectifier current was isolated by subtraction of $K^+$ currents in the presence of a high concentration of TEA (20 mM) from $K^+$ currents in the presence of low TEA (0.5 mM) ($I_{Kslow} = I_{0.5 \text{mM TEA}} - I_{20 \text{mM TEA}}$). The activation curve of $I_{Kslow}$ had a more depolarized midpoint potential ($-5.9 \pm 0.6$ mV) than that of $I_{Kfast}$ and a less steep slope factor ($16.3 \pm 0.6$ mV) than that of $I_{Kfast}$ (Fig. 3C, Table 1, $n = 16$). $I_{Kslow}$ did not completely inactivate, with roughly 10% of current remaining (Fig. 3C). The inactivation curve of $I_{Kslow}$ displayed a midpoint potential ($-60.8 \pm 0.8$ mV) slightly more depolarized than that of $I_{Kfast}$, but a similar slope factor ($26.6 \pm 0.7$ mV) (Fig. 3C, Table 1, $n = 8$). The 20–80% risetime of $I_{Kslow}$ measured between $57$ and $-23$ mV was not dependent on voltage and ranged between $14.6 \pm 4.6$ and $34.3 \pm 6.8$ ms (Fig. 3D, Table 1, $n = 11$). The decay time constant of $I_{Kslow}$ measured at $57$ mV and best fitted with a monoeXponential function, was much slower ($301.6 \pm 109.0$ ms, $n = 6$, Table 1) than that of $I_{Kfast}$ and was independent of voltage (Fig. 3D). The deactivation time constant of $I_{Kslow}$ measured at test pulse to $-43$ mV was slower ($20.4 \pm 5.4$ ms) than that of $I_{Kfast}$ (Fig. 3E, Table 1, $n = 6$).

**FIG. 2.** Properties of fast delayed rectifier $K^+$ currents ($I_{Kfast}$). A: fast delayed rectifier $K^+$ currents from a representative interneuron were isolated by subtraction ($I_{TTX} - I_{0.5 \text{mM TTX}}$) and evoked by test pulses between $57$ and $-73$ mV following a prepulse to $-133$ mV. B: inactivation of $I_{Kfast}$ was studied by applying 1-s prepulses between $-143$ and $27$ mV followed by a test pulse to $57$ mV. C: activation curve of $I_{Kfast}$ (open triangles, $n = 14$) was fitted by a first-order Boltzmann function. Because $I_{Kfast}$ does not completely inactivate during depolarized prepulses, the inactivation curve (open circles, $n = 7$) was fitted by a Boltzmann function plus a constant. D: the 20–80% risetime and decay time of $I_{Kfast}$ were plotted against test pulse potentials ($n = 11$ and 4, respectively). E: the deactivation time course of $I_{Kfast}$ ($I_{TTX} - I_{0.5 \text{mM TTX}}$) was obtained by fitting the decay of the tail current evoked at $-43$ mV with a monoeXponential equation.
A-type K\(^+\) current. A-type K\(^+\) currents were isolated in the presence of a high concentration of TEA (I\(_{20\text{mM,TEA}}\)). The activation curve of A-type K\(^+\) currents had a midpoint potential of \(-4.9 \pm 0.3\) mV, with a slope factor of 18.5 \pm 0.3 mV (Fig. 4C, Table 1, n = 14). The inactivation curve of A-type K\(^+\) currents had a more hyperpolarized midpoint potential (\(-84.1 \pm 0.2\) mV) than that of other currents and a much steeper slope factor (10.0 \pm 0.2 mV) than that of I\(_{\text{Kfast}}\) and I\(_{\text{Kslow}}\) (Fig. 4C, Table 1, n = 6). The 20–80\% risetime of A-type K\(^+\) currents measured between 57 and \(-33\) mV was voltage dependent and varied between 0.6 \pm 0.04 and 2.9 \pm 0.6 ms (Fig. 4D, n = 14). The decay of A-type K\(^+\) currents, measured at a test pulse potential of 57 mV, was best fitted with a biexponential function (\(\tau_{\text{fast}}\): 11.1 \pm 1.6 ms, 54\% contribution; \(\tau_{\text{slow}}\): 46.0 \pm 6.0 ms, 46\% contribution, n = 13). At test pulse potentials ranging between 57 and \(-33\) mV, \(\tau_{\text{fast}}\) and \(\tau_{\text{slow}}\) were independent of voltage (at \(-33\) mV, \(\tau_{\text{fast}}\): 6.4 \pm 2.2 ms; \(\tau_{\text{slow}}\): 38.7 \pm 7.5 ms, Fig. 4E). The recovery from inactivation of A-type K\(^+\) currents, studied with a double-pulse protocol (Fig. 4F), was best fitted by a biexponential function with a \(\tau_{\text{fast}}\) of 29.0 \pm 2.0 ms (63\% contribution) and a \(\tau_{\text{slow}}\) of 141.6 \pm 34.5 ms (15\% contribution) (Fig. 4F, Table 1, n = 10).

\(\alpha\)-DTX-sensitive K\(^+\) current. A fourth K\(^+\) current was isolated in another interneuron. The activation curve of K\(^+\) currents in the presence of \(\alpha\)-DTX was fitted by a Boltzmann function plus a constant (\(I_{\text{D}} = I_{\text{TTX}} - I_{\text{D,TXTX}}\)). This \(\alpha\)-DTX-sensitive K\(^+\) current, described as I\(_{\text{D}}\), in other cortical neurons (Bekkers and Delaney 2001; Locke and Nerbonne 1997; Storm 1988), was present in 75\% of LM/RAD-INSs (Figs. 1E and 5A). The activation curve of I\(_{\text{D}}\) had a midpoint potential of \(-3.8 \pm 0.8\) mV and a much less steep slope factor (24.9 \pm 0.7 mV) than that of other currents (Fig. 5B, Table 1, n = 9). Because the I\(_{\text{D}}\) current obtained by subtraction was of small amplitude, its inactivation curve could not be fully characterized. The 20–80\% risetime of I\(_{\text{D}}\), measured at 57 mV, was 4.4 \pm 1.9 ms. Its decay was best fitted with a monoexponential function and was 49.3 \pm 5.0 ms (Table 1, n = 9).

In other cortical neurons (Bekkers and Delaney 2001; Locke and Nerbonne 1997) the \(\alpha\)-DTX-sensitive I\(_{\text{D}}\) was also found to be blocked by low concentrations of 4-AP. Therefore we also isolated the low 4-AP-sensitive K\(^+\) currents I\(_{\text{D,4AP}}\) by subtracting...
K⁺ currents in the presence of low 4-AP (60 μM) from K⁺ currents in the presence of TTX and TEA (Iₒ = IₒTTX/TEA - IₒTTX/TEA +4-AP) and compared their properties with α-DTX-sensitive K⁺ current (Fig. 5, C and D).

When K⁺ currents were recorded in TTX and low TEA (0.5 mM) to block the I_k fast current, which is also sensitive to low concentrations of 4-AP (Lien et al. 2002), 60 μM 4-AP significantly reduced total K⁺ currents (TTX/TEA = 937.7 ± 166.9 pA; +4-AP = 700.5 ± 158.6 pA; blocked fraction = 0.27 ± 0.04; Fig. 5D, n = 4, P < 0.05). This value is similar to the blocked fraction of the total K⁺ current by α-DTX (TTX = 741.2 ± 126.5 pA; +α-DTX = 592.6 ± 120.8 pA; blocked fraction = 0.25 ± 0.04; Fig. 5D, n = 12, P < 0.05). When K⁺ currents were recorded in TTX and high TEA (20 mM), the application of 60 μM 4-AP also reduced K⁺ currents (TTX/TEA = 379.2 ± 95.8 pA; +4-AP = 317.1 ± 96.5 pA; blocked fraction = 0.19 ± 0.06; Fig. 5D, n = 3, P < 0.05). Moreover, K⁺ current sensitive to low concentrations of 4-AP isolated by subtraction (Fig. 5C, inset) had a risetime similar to that of Iₒ (4.4 ± 1.9 ms for Iₒ; 5.3 ± 3.2 ms for 4-AP-sensitive current isolated in low
TEA; 3.3 ± 1.5 ms for 4-AP-sensitive current isolated in high TEA; Fig. 5D). Similarly, the decay time constants of K+ currents sensitive to 4-AP isolated in low and high TEA were not different from those of $I_D$ (49.3 ± 5.0 ms for $I_D$; 40.7 ± 15.2 ms for K+ currents sensitive to low concentrations of 4-AP isolated in low TEA; 66.1 ± 10.9 ms for K+ currents sensitive to low concentrations of 4-AP isolated in high TEA; Fig. 5D). These results indicate that the majority of LM/RAD-INs express $I_D$ and a K+ current that is sensitive to low concentrations of 4-AP. Taken together, our experiments indicate that four distinct subtypes of K+ currents activated at near threshold membrane potentials can be differentiated in LM/RAD-INs based on their sensitivity to TEA, 4-AP, and α-DTX, as well as their gating properties.

Role of $I_D$ in membrane potential oscillations. Previous work has shown that A-type K+ currents contribute to MPOs in LM/RAD-INs, whereas TEA-sensitive K+ currents are not necessary (Chapman and Lacaille 1999a). Since MPOs and $I_D$ are similarly sensitive to low concentrations of 4-AP, we investigated whether $I_D$ contributes to MPOs in LM/RAD-INs by comparing the effects of α-DTX and low concentrations of 4-AP on MPOs in current-clamp recordings. Membrane potential oscillations were characterized near the action potential threshold in the presence of N-methyl-D-aspartate (NMDA), non-NMDA, and GABA A receptor antagonists d-2-amino-5-phosphonopentanoic acid [D-AP5], 50 μM; (6-cyano-7-nitroquinoxaline-2,3-dione [CNQX], 20 μM; bicuculline, 25 μM; Fig. 6, A and C) (Bourdeau et al. 2007; Chapman and Lacaille 1999a). The mean power of MPOs was significantly reduced in the presence of α-DTX (1.1 ± 0.1 mV2/Hz in control vs. 0.6 ± 0.1 mV2/Hz in α-DTX; Fig. 6, A, B, and E, n = 7, P < 0.05). In contrast, the peak frequency of MPOs was unchanged in α-DTX (1.8 ± 0.1 Hz in control vs. 2.0 ± 0.1 Hz in α-DTX; Fig. 6E). Similarly, low concentrations of 4-AP (60 μM) reduced the mean power of MPOs (1.2 ± 0.3 mV2/Hz in control vs. 0.9 ± 0.2 mV2/Hz in 4-AP; Fig. 6, C–E, n = 5, P < 0.05) without changing the peak frequency (2.0 ± 0.3 Hz in control vs. 2.2 ± 0.1 Hz in 4-AP; Fig. 6E). Overall these results indicate that $I_D$ partially contributes to the generation of MPOs in LM/RAD-INs. The observations that MPOs were only partially blocked in the presence of dendrotoxin or low
4-AP are consistent with the known contribution of other currents such as A-type K\(^+\) currents in MPOs (Bourdeau et al. 2007).

**Currents implicated in MPOs: modeling study**

To understand how the complement of K\(^+\) currents identified in LM/RAD-INs contributes to the generation of MPOs, a mathematical single-compartment model of a LM/RAD-IN was built. The model included not only all four potassium currents characterized earlier, but also the persistent and transient sodium currents, leak currents, noise (assumed to be white), and a current-clamp recording from an LM/RAD-IN are shown in Fig. 7. Albeit by driving the model currents with sinusoidal voltages for values in the subthreshold range. This was done computationally by driving the model currents with sinusoidal voltages for five different levels of noise were preserved for these different current levels. The MPO amplitude is not attributable to different noise levels.

**Analysis of the model system**

To dissect out the contribution of the different currents underlying MPOs, we determined the response characteristics of the model system to a noise input by computing its impedance over a range of frequencies and membrane potential values in the subthreshold range. This was done computationally by driving the model currents with sinusoidal voltages for a range of frequencies and membrane potential values in the subthreshold range. The resulting current responses to this drive can be manipulated algebraically to give an expression for the voltage response of the system to a sinusoidal current at a particular frequency (the system’s impedance), which we refer to as the response function of the system. So long as the system is in the subthreshold voltage range and the deviations away from resting potential induced by the current noise are small (on the order of 1 mV, to allow linearity to hold), the response function describes how the spectrum of the membrane potential depends on the spectrum of current noise. In other words, the response function shows where the model system is still present. It also exhibited this voltage-dependent characteristic. The voltage dependence is shown in Fig. 8 for five different levels of injected DC current. Because the intensity and instantiation of the noise were preserved for these different current levels, the MPO amplitude is not attributable to different noise levels.
In Fig. 8 we show that the response function for our model system was able to capture frequency and voltage ranges where subthreshold oscillations occur in the model. Figure 8A illustrates a series of membrane potential traces that approach threshold by increasing the level of DC current while preserving the intensity and instantiation of the noise. Figure 8B shows the model response function near threshold. Warmer colors indicate a greater impedance magnitude. Dashed lines indicate membrane potential values (ΔV = 66.5, 66.0, 65.5, 65.0, and 64.5 mV, in depolarizing order), corresponding to subthreshold traces in A (in blue). Immediately below threshold (ΔV = 64.5 mV) the response function is bimodal: there is a large peak in response near 7 Hz and an additional increase in response toward lower frequencies. Note that the graph is meaningful only below the action potential threshold; above threshold the system exhibits sustained action potentials and this analysis does not apply. Figure 8C shows spectra of predicted and computed traces. Red traces correspond to predicted amplitude spectrum of the subthreshold traces in A, calculated using the response function in B and the particular instantiation of white noise. Blue traces indicate actual spectra computed from simulations. Dashed line indicates amplitude spectra scaled by the mean amplitude of the white noise spectrum. Computed and predicted traces are averaged over eight 2-s intervals. Parameter values as in APPENDIX Table A1; $I_{\text{app}} = 6.8289 \mu A/cm^2$.

In Fig. 8 we show that the response function for our model system was able to capture frequency and voltage ranges where subthreshold oscillations occur in the model. Figure 8A illustrates a series of membrane potential traces that approach threshold by increasing the level of DC current while preserving the intensity and instantiation of the noise. Figure 8B shows
the response function of the system. Note that this involved a summing of the current responses (shown in Supplemental Fig. S1), as described in METHODS. The response function indicates for which frequencies ($x$-axis) and levels of depolarization ($y$-axis) the response (impedance) of the system is expected to be greatest (warmer colors indicate a larger response). The color map is saturated at dark red, with the response function becoming unbound at its peak. This is where the model system became unstable and above which action potentials occurred. The levels of depolarization corresponding to the blue traces in Fig. 8A are shown by dashed lines in Fig. 8B. Note that the DC current can be set so that the system is below threshold but action potentials can still occur because of perturbation by noise. This is seen in the black trace in Fig. 8A (in which the action potential has been truncated), resulting from a slight increase in DC current with respect to the most depolarized of the blue traces.

Figure 8C demonstrates that the response function accurately captures the subthreshold dynamics of the system. The blue traces correspond to the amplitude of the spectra for each of the five subthreshold traces in Fig. 8A, averaged across eight 2-s intervals. The red traces correspond to the predicted amplitude based on the response function by multiplying the Fourier transform of the noise instantiation by the corresponding response function values. The agreement is good and begins to diverge from the actual values only near threshold. The black dashed lines correspond to the response function amplitude scaled by the mean power of white noise used.

Thus the response function represents a mechanistic description of MPO generation and provides additional advantages over simulations alone. First, it indicates where in the frequency range and at what depolarization MPOs are expected. Second, because it does not depend on a particular instantiation of noise (as the simulations would), the results are more general. Finally, it is computationally efficient because the response function can be easily recomputed for different maximal conductance values from the current responses.

**Contribution of the different currents using the response function analysis**

We examined next how the response function is affected by changes in the underlying model currents. We expect the A-type current to have an important effect on MPOs because this has been found in the previous experimental studies (Bourdeau et al. 2007; Chapman and Lacaille 1999a). Indeed, we found that manipulation of the A-type $K^+$ and persistent Na$^+$ currents had a significant effect on the response function (Fig. 9). We show in Supplemental Fig. S2 that changes in the fast and slow delayed rectifiers and the transient sodium and leak currents did not significantly affect the response function—i.e., MPOs are not dependent on these currents. However, the response function was sensitive to manipulations of $I_D$ (see Supplemental material).

In Fig. 9 we investigated how $I_{A}$ and $I_{NaP}$ contribute to MPO generation. We show response functions for a larger range of...
membrane potential values over which maximal conductances of \(I_A\) and \(I_{NaP}\) have been changed. In examining the effects of changes of maximal conductances on the response function, we focused on two properties: the frequency of the most hyperpolarized impedance peak and the sharpness of this peak (whether it occurred abruptly or gradually with depolarization, as can be observed via the color grading). Eliminating the contribution of \(I_A\) caused a reduction in frequency of the peak impedance and a more gradual increase relative to control (Fig. 9, top middle). In contrast, increasing \(I_A\) shifted the frequency of the nonzero peak to higher frequencies and resulted in a sharper rate of increase of impedance around this frequency with depolarization relative to control (Fig. 9, bottom middle). Moreover, changes of maximal conductances of \(I_{NaP}\) had effects opposite to those of \(I_A\) on the response function. A decrease in the maximal conductance of \(I_{NaP}\) caused an increase in peak frequency and a sharpening of the peak (Fig. 9, top right), whereas an increase in \(I_{NaP}\) caused a reduction in peak frequency and a more gradual increase to the peak (Fig. 9, bottom right). This former observation can be seen by the larger color variation (for the same voltage range) in the response function plot.

We also compared the subthreshold amplitude spectrum in the control condition with that of the cases where \(I_A\) and \(I_{NaP}\) were reduced (Fig. 9, bottom left). In both cases the spectra were taken at \(<1\) mV below the impedance peak (~0.5 mV below spike threshold where spiking was first observed to occur). The impedance peak is taken as the most hyperpolarized local maximum of threshold where spiking was first observed to occur). The impedance peak frequency was shifted above the theta-frequency range. When the persistent Na\(^+\) current was inhibited, the peak frequency was shifted above the theta-frequency range and the response function increased more sharply as the system was brought toward threshold. Because the range over which the response function was rapidly increasing shrinks with respect to the control case for a fixed distance away from the peak on the y-axis, the value of the response function was larger for the control case for a fixed distance away from the peak on the control condition (inset, green trace) and a 50% reduction of \(I_{NaP}\) (inset, red trace) both significantly reduced the amplitude of MPOs produced in the control condition (inset, blue trace).

In summary, the persistent Na current is important in maintaining a gradual slope toward impedance peaks and the A-type current is important for keeping impedance values high in the theta-frequency range. This is achieved because of the characteristics of these currents: frequency-dependent current response for A-type current and significant current response changes with depolarization for persistent Na current (see Supplemental Fig. S1).

**Discussion**

Our major findings are that four voltage-gated K\(^+\) currents can be differentiated in LM/RAD-INs based on their pharmacology and activation and inactivation properties: a fast delayed rectifier K\(^+\) current (\(I_{Kfast}\)), a slow delayed rectifier current (\(I_{Kslow}\)), a rapidly inactivating A-type K\(^+\) current (\(I_A\)), and a slowly inactivating K\(^+\) current (\(I_p\)). In addition, A-type K\(^+\) currents contributed predominantly to the total K\(^+\) currents followed by \(I_{Kfast}, I_{Kslow}\), and \(I_p\). Moreover, a single-compartment computational model, based on the experimental data and incorporating transient and persistent Na\(^+\) currents, was sufficient to enable voltage-dependent membrane potential oscillations (MPOs) and demonstrated that A-type K\(^+\) currents modulate the frequency of MPOs and regulate the voltage range over which they occur.

**A-type K\(^+\) currents are predominant in LM/RAD-INs**

Voltage-gated K\(^+\) channels composed of specific Kv subunits shape interneuron firing properties. Kv3-mediated fast delayed rectifier K\(^+\) currents are prevalent in basket cells and OA-INs and generate fast spiking (Lien et al. 2002; Martina et al. 1998; Rudy and McBain 2001). In contrast, Kv4.3-mediated A-type K\(^+\) currents are predominant in LM/RAD-INs and contribute to regular firing (Bourdeau et al. 2007; Chen and Wong 1991; Rudy 1988). Distinct expression of Kv channels also contributes to intrinsic rhythmic activity in specific interneurons because Kv4.3-mediated A-type K\(^+\) currents are necessary for MPOs in LM/RAD-INs (Bourdeau et al. 2007). Interestingly, we found here that LM/RAD-INs also display slowly inactivating \(I_p\) currents, as do CA1 pyramidal cells (Golding et al. 1999; Metz et al. 2007; Storm 1988; Wu and Barish 1992), but unlike CA1 OA-INs and dentate gyrus basket cells (Lien et al. 2002; Martina et al. 1998). Moreover, we found that \(I_p\) currents contribute in part to MPOs in LM/RAD-INs, consistent with the reported sensitivity of MPOs (and of \(I_p\)) to low concentrations of 4-AP (Chapman and Lacaille 1999a). Given that the contribution of individual currents to the total K\(^+\) currents was different in LM/RAD-INs (\(I_A > I_{Kfast} > I_{Kslow} = I_p\)) and in OA-INs (\(I_{Kfast} > I_{Kslow} > I_A\)), our findings suggest that the expression of K\(^+\) channel complements differs between interneuron subgroups and the predominant expression of \(I_A\) and \(I_p\) may define a participation of interneuron subgroups in hippocampal rhythmic activity.

**MPO generation in LM/RAD-INs**

A GENERIC MECHANISM. Based on the physiological characterization of K\(^+\) currents from LM/RAD-INs, we developed a single-compartment model of LM/RAD-INs that incorporates a noise term that is assumed to be due to intrinsic processes such as channel gating. The LM/RAD-IN model exhibits MPOs in the theta-frequency range when depolarized near action potential threshold. Moreover, MPOs show voltage dependence and increase in amplitude with depolarization, as observed experimentally. The generation of MPOs in our model is fully accounted for by the physics concept of critical slowing down, suggesting that this concept accounts for MPOs in LM/RAD-INs.

Critical slowing down (or, simply, critical slowing) consists of the increase in response of a system to a noise input as the system is brought toward an instability or a bifurcation point (a point at which there is a qualitative change in the dynamic output of the system) by slowly varying a parameter of the system. The enhancement in response is preferential, occurring at a frequency close to 0 Hz for an integrator type model system (that occurs with a saddle node type bifurcation) or some positive value for a resonator type model system (that occurs with an Andronov–Hopf type bifurcation; Izhikevich 2007). Depending on the type of bifurcation, the amplitude of peak frequency response as the membrane potential is varied in the subthreshold range follows a different scaling law, with the resonator type being sharper than the integrator type (Steyn-
Ross et al. 2006). The height of the spectral peak at this frequency increases and the autocorrelation of the system’s trajectory broadens (perturbations applied to the system show a slower rate of decay) as the system approaches threshold. Critical slowing has been demonstrated in a variety of systems, including resonant and integrator type model neurons (Steyn-Ross et al. 2006), speech perception (Lancia et al. 2008), human posture (Bardy et al. 2002), ecology (Gandhi et al. 1998), and various physical and chemical systems (Kostko et al. 2007; Oh et al. 2004; Reis and Mullin 2002).

Previous characterization of LM/RAD-INs indicated that the membrane potential response to brief, depolarizing current pulses shows a biphasic exponential decay consisting of an initial rapid phase and a later slower component that is voltage-dependent (Williams et al. 1994; see Fig. 6). This biphasic response first appears at potentials 10–20 mV below spike threshold. As one approaches spike threshold, the time constant and relative contribution of the slow component increase. This describes the prototypical critical slowing phenomenon, in which the component of the system that gives rise to the instability shows a slower response as the system approaches that instability, whereas other components of the system show little change with distance from threshold. Thus in our LM/RAD-IN model system, the enhanced sensitivity to noise near threshold (as given by the increase in amplitude of the MPOs) may be a manifestation of a generic property of excitable systems with noise: critical slowing. Although critical slowing is generic, the details of the LM/RAD-IN model determine its characteristics, such as the voltage range over which its contribution is significant and the frequency around which it is centered.

SPECIFIC CURRENTS AND IMPLICATIONS. Our model shows that $I_{Na}$, $I_{Kfast}$, $I_{Kslow}$, and $I_D$ conductances are all active during subthreshold oscillations. We used a membrane resonance analysis to predict the response of the model system to noise over a range of subthreshold membrane potentials and frequencies. This avoids the need for repeated model simulations for changes in depolarization and/or current balances, which are time-consuming and dependent on the specific instantiation of noise used. Through this analysis we showed that the frequency of MPOs depends largely on the balance of the A-type $K^+$ and the persistent $Na^+$ currents. In fact, our model suggests that, at subthreshold membrane potentials, A-type $K^+$ channels are actively contributing to MPO generation. As the cell approaches spike threshold, the balance of currents shifts toward contributions from $Na^+$ currents, with $I_{Na,p}$ playing an essential role in bringing about the occurrence of MPOs.

The rate and range of the model behavior as it approaches threshold are important aspects of the critically slowed system to consider. In particular, for MPOs to occur there needs to be a gradual rise in the response function as threshold is approached. Changes that make this transition more gradual will extend the range over which critical slowing occurs. This is the case for increasing $I_{Na,p}$ but not for increasing $I_A$ (see response functions of Fig. 9). This suggests that $I_{Na,p}$ plays an essential role to bring about the occurrence of MPOs. This is in contrast to the transient $Na^+$ current, with its faster kinetics and more depolarized activation curve, that does not affect the response function. Similarly, subthreshold oscillations in stellate cells of medial entorhinal cortex are known to depend on persistent $Na^+$ currents for their generation (Alonso and Llinás 1989; Klink and Alonso 1993).

Although we have shown that the theta-frequency MPOs in LM/RAD-INs can arise without the need for any special voltage or frequency dependence of the noise itself, it is likely that such effects do contribute. In stellate cells of the entorhinal cortex, it was shown—through pharmacological block of persistent sodium channels and reinsertion of “virtual” versions of them using dynamic clamp—that subthreshold oscillations in those cells depend critically on the noise contributed by the channels, which activates together with the current in the subthreshold range. Reinserting a purely deterministic version of the channels (i.e., without noise) was insufficient to recover oscillations (Dorval Jr and White 2005). To explicitly verify the contribution of particular currents to critical slowing in LM/RAD-INs, one could take advantage of the ability of dynamic clamp to add or remove the deterministic component of a current (given an adequate model), while leaving the noise level of the system intact. Properties of the critically slowed component of the system could then be directly augmented and the effect on MPOs assessed. Verification of changes in the critically slowed component of the system could be measured by averaging together the subthreshold response to brief current pulses.

Functional significance

We have shown that impedance analysis is sufficient to account for the generation of MPOs in LM/RAD-INs, implying that our linear approximation is valid. For this analysis approach to provide insight, it is important that the determination of MPOs by the underlying currents also governs spiking behavior. There is disagreement over whether related phenomena such as spike timing and reliability depend critically on nonlinear current activity (see Haas and White 2002; Schreiber et al. 2004). Schreiber et al. (2009) recently described distinct spike time reliability behavior for spiking below threshold (driven by current noise) and above threshold (driven by the current mean). For spiking driven by strong noise far below threshold, linear impedance analysis is insufficient to account for reliability behavior and a direct assessment of nonlinear current activation is required. However, close to threshold the linear approximation is valid.

Our results uncover mechanisms of intrinsic rhythmic activity in inhibitory interneurons that may contribute to hippocampal theta activity (Buzsáki 2002). Hippocampal and entorhinal theta activity are in part driven by extrinsic inputs from the medial septum (Mitchell et al. 1982; Petsche et al. 1962). In CA1 pyramidal cells, theta-frequency MPOs generated by intrinsic membrane conductances also contribute to theta activity (Leung and Yim 1991), in addition to their intrinsic resonant oscillatory properties dependent on $I_{Na,p}$, $I_h$, and $I_D$ (Hu et al. 2002). Hippocampal inhibitory interneurons play an additional crucial role in theta activity by rhythmically inhibiting pyramidal cells (Fox 1989; Leung 1984; Ylinen et al. 1995). Septal cholinergic afferents target pyramidal cells and inhibitory interneurons (Léránth and Frotscher 1987), whereas septal GABAergic inputs contact mostly interneurons (Frendt and Antal 1988; Gulyás et al. 1990). Thus during theta activity, activation of septal GABAergic afferents disinhibits pyramidal cells by inhibiting tonically active interneurons (Toth et al. 1997). Rhythmic activation of presynaptic basket and axo-axonic
interneurons, phase-locked with CA1 pyramidal cell firing, may thus contribute to subthreshold membrane potential oscillations in principal cells (Cobb et al. 1995). Finally, rhythmic activity of interneurons driven by intrinsic MPOs may also contribute to hippocampal theta since pyramidal cells can be paced by rhythmic inhibition generated by L/M/RAD interneurons at theta frequency (Chapman and Lacaille 1999a,b). Thus our findings uncover how a differential expression of a complement of K⁺ and Na⁺ channels underlies intrinsic rhythmic activity in CA1 inhibitory interneurons that may contribute to hippocampal theta activity.

APPENDIX

Fast and slow delayed rectifiers, \( I_D \)

The fast and slow delayed rectifier potassium currents are modeled by the Hodgkin–Huxley (HH) type representation

\[
I = \bar{g}M(V - E_K)
\]

where \( \bar{g} \) is the maximal conductance in mS/cm², \( M \) and \( H \) are activation and inactivation gating particles respectively, \( V \) is the membrane potential, and \( E_K \) is the potassium reversal potential. The evolution of the gating particles is given by

\[
\frac{dM}{dt} = \frac{M_a(V) - M}{\tau_M(t)} \quad M_a(V) = \frac{1}{1 + e^{-(V-V_{c1})^2/2\sigma_1^2}}
\]

\[
\frac{dH}{dt} = \frac{H_a(V) - H}{\tau_H(t)} \quad H_a(V) = \frac{A}{1 + e^{(V-V_{c2})^2/2\sigma_2^2}} + (1 - A)
\]

where \( A \) represents the fraction of channels that show inactivation, which is incomplete for the delayed rectifiers. The time constants of inactivation are obtained by averaging together experimental values at all membrane potential values for which it was measured.

Characterization of the \( I_{DTX} \) inactivation steady-state curve was not possible in LM/RAD-INs. The inactivation seen on depolarization to 57 mV is comparable to the \( \alpha \)-DTX-sensitive current described in Guan et al. (2006) in pyramidal cells of the neocortex. The inactivated component of this current is small in the subthreshold range of values of LM/RAD-INs and therefore we model \( I_{DTX} \) as noninactivating, with the understanding that this approximation is appropriate only for subthreshold activity and not necessarily during action potentials.

All other values characterizing activation and inactivation are the values obtained experimentally. Time constants are constant values as determined from the data and are not functions of voltage attributed to the lack of data in this respect. Parameter values are provided in Table A1.

A-type \( K^+ \) current

We model the A-type \( K^+ \) current as a multistate model rather than as an HH model because it is known to be encoded by Kv4.3 in LM/RAD cells based on siRNA experiments (Bourdeau et al. 2007) and detailed models of this current have been developed previously that we can use to further constrain the added parameters. Our A-type model is based on a previously developed model of Kv4.3 (Wang et al. 2004, 2005), but we use a simplified inactivation process. The gating scheme is expressed as

\[
C_0 \xrightarrow{\alpha} C_1 \xrightarrow{\beta} C_2 \xrightarrow{\alpha} C_3 \xrightarrow{\beta} C_4 \xrightarrow{\alpha} O \xrightarrow{\beta} I
\]

The current is given by

\[
I_A = \bar{g} \left( \frac{O}{C_0 + C_1 + C_2 + C_3 + C_4 + I + O} \right) (V - E_K)
\]

The fraction of channels in each state is taken to be continuous and the time evolution of the system is described by

\[
\begin{align*}
C_0' &= C_1 * \beta(V) - C_0 * \alpha(V) \\
C_1' &= C_0 * 4 * \alpha(V) - C_1 * \beta(V) + C_2 * 2 * \beta(V) - C_1 * 3 * \alpha(V) \\
C_2' &= C_1 * 3 * \alpha(V) - C_2 * 2 * \beta(V) + C_3 * 3 * \beta(V) - C_2 * 2 * \alpha(V) \\
C_3' &= C_2 * 2 * \alpha(V) - C_3 * 3 * \beta(V) + C_4 * 4 * \beta(V) - C_3 * \alpha(V) \\
O' &= C_4 * K_1 - O * K_2 + K_0 * I - K_f * O \\
I' &= K_f * O - K_b * I
\end{align*}
\]

where the voltage-dependent functions alpha and beta are given by

\[
\alpha(V) = f_\alpha \left( a_1 \exp(z_{a1} e_0 VF/RT) \exp((V + 10)/10.0) + a_2 \exp(z_{a2} e_0 VF/RT) \right)
\]

\[
\beta(V) = f_\beta \left( b_1 \exp(z_{\beta1} e_0 VF/RT) \exp((V + 5)/10.0) + b_2 \exp(z_{\beta2} e_0 VF/RT) \right)
\]

with

\[
\begin{align*}
f_\alpha &= 1/\left(1 + \exp(V + 10.0)/10.10\right) \\
f_\beta &= 1/\left(1 + \exp(V + 5.0)/10.00\right)
\end{align*}
\]

The functions \( \alpha(V) \) and \( \beta(V) \) are chosen so that they approach simple exponential functions for depolarized and hyperpolarized membrane potentials

\[
\alpha(V) \approx \exp(z_{a1} e_0 VF/RT) \quad \alpha(V) \approx \exp(z_{a2} e_0 VF/RT)
\]

\[
\beta(V) \approx \exp(z_{\beta1} e_0 VF/RT) \quad \beta(V) \approx \exp(z_{\beta2} e_0 VF/RT)
\]

when \( V \) approaches +50 mV when \( V \) approaches −120 mV

Values for the effective gating charges \( z_{\alpha1}, z_{\alpha2}, z_{\beta1}, z_{\beta2} \) and \( e_0 \) are fit to the L/M/RAD-IN \( I_A \) current steady-state curve, with values as given in Table A1.

Inactivation in L/M/RAD-INs follows a biexponential function (with fast and slow time constants of 11.1 and 46.0 ms, respectively). Examination of the unaveraged steady-state inactivation curves from individual cells shows that the half-activation values exhibit high variability (with values spanning ~20 mV), whereas slope factors are relatively constrained. This specific variability can be accounted for by changes in the \( K_f \) and \( K_b \) parameters of the model. When \( K_f \) is much larger than \( K_b \) the inactivation time constant of the model is approximately 1/K_b (our model form exhibits only a single time constant of decay) and \( K_f \) and \( K_b \) together determine \( V_{1/2} \). The values used match the fast component of experimentally characterized inactivation and give a \( V_{1/2} \) value lying in the more depolarized range of the data. Parameters governing activation (such as \( K_f \) and \( K_b \)) were not explored because \( I_{DTX} \) activation in LM/RAD-INs was in good agreement with the model of Wang et al. (2004, 2005).

Transient and persistent sodium currents

The transient sodium current has not been characterized in LM/RAD-INs and we turned to a previously developed model that was used to represent the sodium current in a hippocampal interneuron (Wang and Buzsáki 1996). The current is given by

\[
I_{NaT} = \bar{g}(M_a)^3 H(V - E_{Na})
\]

where \( \bar{g} \) is the maximal conductance, \( H \) is the inactivation gating particle, \( E_{Na} \) is the sodium reversal potential, and \( V \) is the membrane potential. The activation of the channel is assumed to change fast with respect to the other state variables of the system so channel activation is set to its steady-state value \( M_a(V) \), given by

\[
M_a(V) = \frac{\alpha_M(V)}{\alpha_M(V) + \beta_M(V)}
\]
TABLE A1. Symbols, values, units, and descriptions of variables used in this study

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C$</td>
<td>1</td>
<td>$\mu$F cm$^{-2}$</td>
<td>Membrane capacitance</td>
</tr>
<tr>
<td>$E_k$</td>
<td>$-101$</td>
<td>mV</td>
<td>Reversal potential of potassium currents</td>
</tr>
<tr>
<td>$E_{Na}$</td>
<td>$55$</td>
<td>mV</td>
<td>Reversal potential of sodium currents</td>
</tr>
<tr>
<td>$E_L$</td>
<td>$-60$</td>
<td>mV</td>
<td>Reversal potential of leak currents</td>
</tr>
<tr>
<td>Fast delayed rectifier</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g$</td>
<td>4.19</td>
<td>mS cm$^{-2}$</td>
<td>Maximum conductance</td>
</tr>
<tr>
<td>$V_{1/2}$ (activation)</td>
<td>$-14.3$</td>
<td>mV</td>
<td>Membrane potential at half activation</td>
</tr>
<tr>
<td>$k$ (activation)</td>
<td>$10.7$</td>
<td>mV</td>
<td>Slope of steady-state activation at $V_{1/2}$</td>
</tr>
<tr>
<td>$\tau$ (activation)</td>
<td>$10.3$</td>
<td>ms</td>
<td>Time constant of activation</td>
</tr>
<tr>
<td>$V_{1/2}$ (inactivation)</td>
<td>$-64.6$</td>
<td>mV</td>
<td>Membrane potential at half inactivation</td>
</tr>
<tr>
<td>$k$ (inactivation)</td>
<td>$24.5$</td>
<td>mV</td>
<td>Slope of steady-state inactivation curve at $V_{1/2}$</td>
</tr>
<tr>
<td>$\tau$ (inactivation)</td>
<td>$108$</td>
<td>ms</td>
<td>Time constant of inactivation</td>
</tr>
<tr>
<td>$A$</td>
<td>$0.853$</td>
<td>—</td>
<td>Fraction of current inactivating</td>
</tr>
<tr>
<td>Slow delayed rectifier</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g$</td>
<td>2.7</td>
<td>mS cm$^{-2}$</td>
<td>Maximum conductance</td>
</tr>
<tr>
<td>$V_{1/2}$ (activation)</td>
<td>$-5.9$</td>
<td>mV</td>
<td>Membrane potential at half activation</td>
</tr>
<tr>
<td>$k$ (activation)</td>
<td>$16.3$</td>
<td>mV</td>
<td>Slope of steady-state activation at $V_{1/2}$</td>
</tr>
<tr>
<td>$\tau$ (activation)</td>
<td>$20.8$</td>
<td>ms</td>
<td>Time constant of activation</td>
</tr>
<tr>
<td>$V_{1/2}$ (inactivation)</td>
<td>$-60.8$</td>
<td>mV</td>
<td>Membrane potential at half inactivation</td>
</tr>
<tr>
<td>$k$ (inactivation)</td>
<td>$26.6$</td>
<td>mV</td>
<td>Slope of steady-state inactivation curve at $V_{1/2}$</td>
</tr>
<tr>
<td>$\tau$ (inactivation)</td>
<td>$235$</td>
<td>ms</td>
<td>Time constant of inactivation</td>
</tr>
<tr>
<td>$A$</td>
<td>$0.917$</td>
<td>—</td>
<td>Fraction of current inactivating</td>
</tr>
<tr>
<td>$I_D$ current</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g$</td>
<td>2.08</td>
<td>mS cm$^{-2}$</td>
<td>Maximum conductance</td>
</tr>
<tr>
<td>$V_{1/2}$ (activation)</td>
<td>$-3.8$</td>
<td>mV</td>
<td>Membrane potential at half activation</td>
</tr>
<tr>
<td>$k$ (activation)</td>
<td>$24.9$</td>
<td>mV</td>
<td>Slope of steady-state activation at $V_{1/2}$</td>
</tr>
<tr>
<td>$\tau$ (activation)</td>
<td>$4.4$</td>
<td>ms</td>
<td>Time constant of activation</td>
</tr>
<tr>
<td>Transient A-type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{g}$</td>
<td>19.5</td>
<td>mS cm$^{-2}$</td>
<td>Maximum conductance</td>
</tr>
<tr>
<td>$k_1$</td>
<td>$6$</td>
<td>ms$^{-1}$</td>
<td>Pre-open closed state to open state transition rate</td>
</tr>
<tr>
<td>$k_2$</td>
<td>$1.5$</td>
<td>ms$^{-1}$</td>
<td>Open state to pre-open closed state transition rate</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$0.09$</td>
<td>ms$^{-1}$</td>
<td>Forward inactivation rate</td>
</tr>
<tr>
<td>$k_4$</td>
<td>$0.00075$</td>
<td>ms$^{-1}$</td>
<td>Recovery from inactivation rate; when this is much smaller than $k_3$, it determines the steady-state inactivation rate</td>
</tr>
<tr>
<td>$z_{at}$</td>
<td>$0.12$</td>
<td>—</td>
<td>Forward gating charge for $V \rightarrow +50$ mV</td>
</tr>
<tr>
<td>$z_{at}$</td>
<td>$0.5$</td>
<td>—</td>
<td>Forward gating charge for $V \rightarrow +120$ mV</td>
</tr>
<tr>
<td>$z_{m1}$</td>
<td>$-0.34$</td>
<td>—</td>
<td>Reverse gating charge for $V \rightarrow +50$ mV</td>
</tr>
<tr>
<td>$z_{m2}$</td>
<td>$-0.48$</td>
<td>—</td>
<td>Reverse gating charge for $V \rightarrow +120$ mV</td>
</tr>
<tr>
<td>$a_1$</td>
<td>$0.425$</td>
<td>—</td>
<td>Weighting of exponentials</td>
</tr>
<tr>
<td>$a_2$</td>
<td>$0.0836$</td>
<td>—</td>
<td>Weighting of exponentials</td>
</tr>
<tr>
<td>$b_1$</td>
<td>$0.2244$</td>
<td>—</td>
<td>Weighting of exponentials</td>
</tr>
<tr>
<td>$b_2$</td>
<td>$0.0252$</td>
<td>—</td>
<td>Weighting of exponentials</td>
</tr>
<tr>
<td>Transient sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{g}$</td>
<td>$30$</td>
<td>mS cm$^{-2}$</td>
<td>Maximum conductance</td>
</tr>
<tr>
<td>Persistent sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{g}$</td>
<td>$0.6$</td>
<td>mS cm$^{-2}$</td>
<td>Maximum conductance</td>
</tr>
<tr>
<td>$V_{1/2}$ (activation)</td>
<td>$-51$</td>
<td>mV</td>
<td>Half activation</td>
</tr>
<tr>
<td>$k$ (activation)</td>
<td>$5$</td>
<td>mV</td>
<td>Slope of steady-state activation at $V_{1/2}$</td>
</tr>
<tr>
<td>$\tau$ (activation)</td>
<td>$5$</td>
<td>ms</td>
<td>Time constant of activation</td>
</tr>
<tr>
<td>Leak current</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{g}$</td>
<td>$0.04$</td>
<td>mS cm$^{-2}$</td>
<td>Leak conductance</td>
</tr>
</tbody>
</table>

The persistent sodium current, based on the data in French et al. (1990) for hippocampal pyramidal cells, is given by

$$I_{NaP} = \bar{g}P(V - E_{Na})$$

with the activation dynamics of $P$ described by

$$\frac{dP}{dt} = \frac{P_s(V) - P}{\tau}$$

$$P_s(V) = \frac{1}{1 + \exp[-(V - V_{1/2})/k]}$$

We set the transient sodium current maximal conductance value to a value that gives an action potential amplitude of about 20 mV and the persistent sodium current maximal conductance at 1/50th of this value. Parameter values are given in Table A1.
REFERENCES


