

Properties and Role of I_h in the Pacing of Subthreshold Oscillations in Entorhinal Cortex Layer II Neurons

CLAYTON T. DICKSON,¹ JACOPO MAGISTRETTI,² MARK H. SHALINSKY,¹ ERIK FRANSÉN,³ MICHAEL E. HASSELMO,⁴ AND ANGEL ALONSO¹

¹Department of Neurology and Neurosurgery, Montreal Neurological Institute and McGill University, Montreal, Quebec H3A 2B4, Canada; ²Department of Experimental Neurophysiology, Istituto Nazionale Neurologico C. Besta, Milan 510, 20133 Italy; ³Department of Numerical Analysis and Computing Science, Royal Institute of Technology, S-100 44 Stockholm, Sweden; and ⁴Department of Psychology, Boston University, Boston, Massachusetts 02215

Dickson, Clayton T., Jacopo Magistretti, Mark H. Shalinsky, Erik Fransén, Michael E. Hasselmo, and Angel Alonso. Properties and role of I_h in the pacing of subthreshold oscillations in entorhinal cortex layer II neurons. *J. Neurophysiol.* 83: 2562–2579, 2000. Various subsets of brain neurons express a hyperpolarization-activated inward current (I_h) that has been shown to be instrumental in pacing oscillatory activity at both a single-cell and a network level. A characteristic feature of the stellate cells (SCs) of entorhinal cortex (EC) layer II, those neurons giving rise to the main component of the perforant path input to the hippocampal formation, is their ability to generate persistent, Na^+ -dependent rhythmic subthreshold membrane potential oscillations, which are thought to be instrumental in implementing theta rhythmicity in the entorhinal-hippocampal network. The SCs also display a robust time-dependent inward rectification in the hyperpolarizing direction that may contribute to the generation of these oscillations. We performed whole cell recordings of SCs in *in vitro* slices to investigate the specific biophysical and pharmacological properties of the current underlying this inward rectification and to clarify its potential role in the genesis of the subthreshold oscillations. In voltage-clamp conditions, hyperpolarizing voltage steps evoked a slow, noninactivating inward current, which also deactivated slowly on depolarization. This current was identified as I_h because it was resistant to extracellular Ba^{2+} , sensitive to Cs^+ , completely and selectively abolished by ZD7288, and carried by both Na^+ and K^+ ions. I_h in the SCs had an activation threshold and reversal potential at approximately -45 and -20 mV, respectively. Its half-activation voltage was -77 mV. Importantly, bath perfusion with ZD7288, but not Ba^{2+} , gradually and completely abolished the subthreshold oscillations, thus directly implicating I_h in their generation. Using experimentally derived biophysical parameters for I_h and the low-threshold persistent Na^+ current (I_{NaP}) present in the SCs, a simplified model of these neurons was constructed and their subthreshold electroresponsiveness simulated. This indicated that the interplay between I_{NaP} and I_h can sustain persistent subthreshold oscillations in SCs. I_{NaP} and I_h operate in a “push-pull” fashion where the delay in the activation/deactivation of I_h gives rise to the oscillatory process.

INTRODUCTION

The hyperpolarization-activated inward current (I_h ; usually referred to as I_f in heart) has been implicated in the pacemaking of both single-cell and network rhythmicity (for recent reviews, see DiFrancesco 1993; Lüthi and McCormick 1998;

Pape 1996). Typically, this current acts to promote depolarization after a hyperpolarizing event. This, in combination with Ca^{2+} currents, functions to induce low-threshold rhythmic discharge in a number of neurons and thus contributes to brain rhythm generation (Llinás and Jahnsen 1982; Lüthi et al. 1998; McCormick and Pape 1990; Steriade and Llinás 1988; Steriade et al. 1993). In contrast to Ca^{2+} -dependent oscillations, numerous studies have shown that some, mainly cortical, neuronal populations can generate Na^+ -dependent rhythmic subthreshold membrane potential oscillations that are thought also to be implicated in the genesis of cortical rhythms (Alonso and Llinás 1989; reviewed by Connors and Amitai 1997). The role of near-threshold conductances, including I_h , in the generation of these Na^+ -dependent subthreshold oscillatory events is less clear.

A prominent case of Na^+ -dependent subthreshold oscillatory activity is observed in the principal neurons from entorhinal cortex (EC) layer II (Alonso and Klink 1993; Alonso and Llinás 1989). These glutamatergic neurons, named by Cajal as the stellate cells (SCs) (Ramon y Cajal 1902), funnel most of the neocortical input to the hippocampal formation via the perforant pathway (for review, see Dolorfo and Amaral 1998) and appear to be generators of limbic theta rhythm (Alonso and García-Austt 1987a,b; Buzsáki 1996; Dickson et al. 1995). *In vitro* current-clamp studies have shown that the current-voltage relationship of EC layer II SCs is extremely nonlinear, displaying robust inward rectification in both the depolarizing and hyperpolarizing direction. Inward rectification in the depolarizing direction is generated by a persistent subthreshold Na^+ current (I_{NaP}) (Magistretti and Alonso 1999; Magistretti et al. 1999) (for a recent review on I_{NaP} , see also Crill 1996) that has been shown to be necessary for the development of the robust theta frequency subthreshold oscillations that the SCs display (Alonso and Klink 1993; Alonso and Llinás 1989). On the other hand, the time-dependent inward rectification in the hyperpolarizing direction is affected by extracellular Cs^+ but not Ba^{2+} and thus is likely to be generated by the nonspecific cationic current I_h (Klink and Alonso 1993). Given the properties and role of I_h in pacemaking in other excitable cells, it was proposed that this current also could contribute to the genesis of subthreshold oscillations in SCs (Alonso and Llinás 1989; Klink and Alonso 1993; White et al. 1995) although the exact nature of this role was not specified.

Using the whole cell patch-clamp technique in the EC slice

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

preparation, the aim of the present study was to characterize the specific properties of I_h in the SCs and to examine the role of this current in the generation of subthreshold membrane potential oscillations in these cells. In addition, a simplified biophysical simulation based on the voltage- and current-clamp data was used to study the interactions between I_h and I_{NaP} in the generation of such oscillations. Our results indicate that the dynamic interplay between the gating and kinetic properties of I_h and I_{NaP} is essential for the generation of rhythmic subthreshold oscillations by the SCs. Given the key position of the SCs in the temporal lobe memory system, modulation of I_h in the SCs may have major implications for the control of population dynamics in the entorhinal network and in the memory processes it carries out. Some of these results have been presented previously in abstract form (Dickson and Alonso 1996, 1998; Fransén et al. 1998).

METHODS

General

Brain slices were prepared from male Long-Evans rats (100–250 g, i.e., 30–60 days of age) as previously described (Alonso and Klink 1993). Briefly, animals were decapitated quickly, and the brain was removed rapidly from the cranium, blocked, and placed in a cold (4°C) Ringer solution (pH 7.4 by saturation with 95% O_2 -5% CO_2) containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4 , 2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, and 10 glucose. Horizontal slices of the retro-hippocampal region were cut at 350–400 μm on a vibratome (Pelco

Series 1000, Redding, CA) and were transferred to an incubation chamber in which they were kept submerged for ≥ 1 h at room temperature (24°C). Slices were transferred, one at a time, to a recording chamber and were superfused with Ringer solution, also at room temperature. The chamber was located on the stage of an upright, fixed-stage microscope (Axioskop, Zeiss) equipped with a water immersion objective ($\times 40$ –63: long-working distance), Nomarski optics, and a near-infrared charge-coupled device (CCD) camera (Sony XC-75). With this equipment, stellate and pyramidal-like cells could be distinguished based on their shape, size, and position within layer II of the medial entorhinal cortex (Fig. 1A) (Klink and Alonso 1997). Stellate cells (SCs) were selected for whole cell recording.

Recording

Patch pipettes (4–7 M Ω) were filled with (in mM) 140–130 gluconic acid (potassium salt: K-gluconate), 5 NaCl, 2 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES), 0.5 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 2 ATP (ATP Tris salt), and 0.4 GTP (GTP Tris salt), pH 7.25 with KOH. In additional experiments performed to assess the contribution of chloride ions to I_h , a modified intracellular solution was made containing (in mM) 120 K-gluconate, 10 KCl, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 0.5 EGTA, 2 ATP-Tris, and 0.4 GTP-Tris, pH 7.25 with KOH. The liquid junction potential was estimated following the technique of Neher (1992). In brief, the offset was zeroed while recording the potential across the patch pipette and a commercial salt-bridge ground electrode (MERE 2, WPI, Sarasota, FL) when the chamber was filled with the same intracellular solution as used in the pipette. After zeroing, the chamber solution was

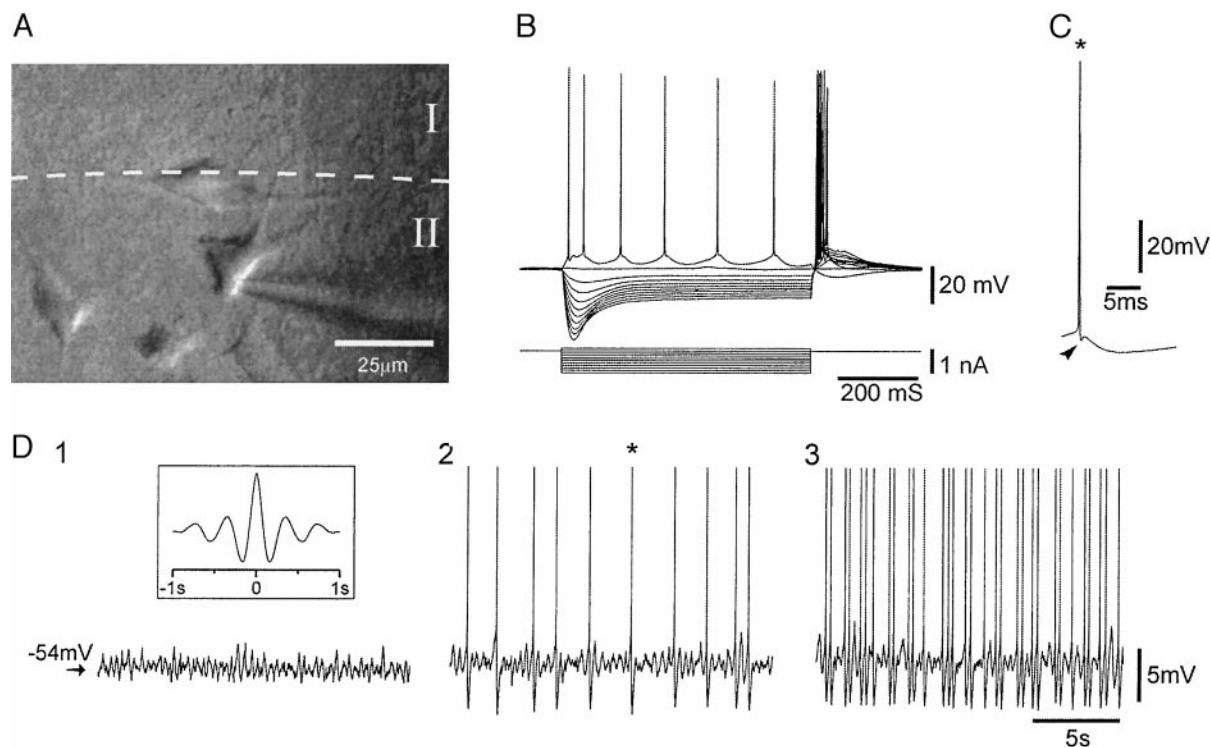


FIG. 1. Basic electrophysiological profile of entorhinal cortex (EC) layer II stellate cells (SCs) under whole cell current-clamp recording conditions. A: digitized photomicrograph demonstrating the visualization of a patched SC. ---, approximate border between layers I and II. B: V-I relationship of the SC in A demonstrating robust time-dependent inward rectification in the hyperpolarizing direction. C: action potential from (D2) (*) at an expanded time and voltage scale. Note the fast-afterhyperpolarization/depolarizing afterpotential/medium afterhyperpolarization (fast-AHP/DAP/medium-AHP) sequence characteristic of these cells. D: subthreshold membrane potential oscillations (1 and 2) and spike clustering (3) develop at increasingly depolarized membrane potential levels positive to about -55 mV. Autocorrelation function (inset in 1) demonstrates the rhythmicity of the subthreshold oscillations.

replaced with the extracellular recording solution, and the potential recorded was used as an estimate of the liquid junction potential. Using this method, we recorded a value between 2 and 3 mV. Membrane potential values reported herein do not contain this correction.

Tight seals (>1 G Ω) were formed on cell bodies of selected EC layer II SCs, and whole cell recordings were made by rupturing the cell membrane with negative pressure. Both current- and voltage-clamp recordings were made with an Axopatch 1D patch-clamp amplifier (Axon Instruments, Foster City, CA). For current-clamp recordings, the low-pass filter (-3 dB) was set at 10 kHz, whereas for voltage clamp, it was set at 2 kHz. All current- and some voltage-clamp experiments were stored by PCL coding on VHS tape (Neurocorder, Neurodata, New York), and all voltage-clamp experiments were stored on computer by digital sampling at 4 kHz, using pClamp software (V6.0, Axon Instruments). Data stored on VHS tape was digitized and plotted off-line by sampling at 20 kHz using Axoscope software (V1.1, Axon Instruments).

The identification of neurons as SCs was confirmed by current-clamp recordings demonstrating the presence of robust inward rectification with hyperpolarizing current pulses in addition to the presence of subthreshold membrane potential oscillations at depolarized levels (Fig. 1, B and C) (Alonso and Klink 1993). SCs fulfilling the following criteria were considered acceptable for further analysis: stable membrane potential less than -50 mV, input resistance >75 M Ω , overshooting spike, and a balanced series resistance <20 M Ω compensated between 60 and 80%.

Solutions

Various salts and drugs were added directly to the perfusate from concentrated stock solutions during experiments. Divalent cations such as Ba^{2+} , Co^{2+} , or Cd^{2+} were added to a modified Ringer solution without phosphates or sulfates. To isolate I_h to compute activation curves using the tail current method (see RESULTS), the following solution was used (in mM): 80 NaCl, 40 tetraethylammonium chloride (TEA-Cl), 5 KCl, 4 4-aminopyridine (4-AP), 2 MgCl_2 , 2 BaCl_2 , 2 CoCl_2 , 1 CaCl_2 , 0.2 CdCl_2 , 26 NaH_2CO_3 , and 10 glucose and 1 μM tetrodotoxin (TTX). Lowering the extracellular concentration of sodium ions (NaCl) was achieved using equimolar *N*-methyl-D-glucamine substitution in a no phosphate/sulfate Ringer solution. Alterations in the concentration of potassium ions (KCl) was achieved in the same way in a no phosphate/sulfate Ringer solution with a consistent concentration (119 mM) of NaCl. To prevent the influence of synaptic transmission on the subthreshold membrane behavior in current-clamp recordings, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX: 10 μM), DL-2-amino-5-phosphonopentanoic acid (AP-5: 50 μM), bicuculline methiodide (BMI: 10 μM), and 2-hydroxysaclofen (2-OH saclofen: 100 μM) were added to the Ringer solution. To block I_h , CsCl (1–6 mM) or ZD7288 (100 μM) was added directly to the Ringer solution. All salts were purchased from BDH (Toronto, CA), whereas TEA-Cl, 4-AP, TTX, and BMI were purchased from Sigma (St. Louis, MO). CNQX, AP-5, 2-OH-saclofen, and ZD7288 were purchased from Tocris Cookson (UK).

Analysis

Traces were plotted and measurements made with the use of pClamp (Clampfit) and Origin (Microcal, Northampton, MA) software packages. Curve fitting of subtracted traces was conducted with pClamp software. The fittings were made from a time point 15 ms after the application of the hyperpolarizing voltage step so as to minimize any capacitive or membrane charging transients. All curve-fitting procedures were optimized using the least sum of squares method. The standard deviation between the fit and the data were used to estimate the goodness of the fit. Autocorrelational analysis was conducted with Matlab (Mathworks, Natick, MA). Spectral (Fourier) analysis was conducted using both Origin and Matlab.

Biophysical simulation

A simple Hodgkin-Huxley model was assumed for describing I_h activation and deactivation. We applied the basic relationship

$$I_h(V, t) = G_h(V, t) \cdot (V - V_h) \quad (1)$$

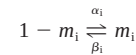
where

$$G_h(V, t) = G_{h\text{Max}} \cdot m(V, t) \quad (2)$$

and m is the probability of the activating particle to be in the permissive position. Because I_h activation and deactivation could be properly fitted with double-exponential functions, Eq. 2 was applied separately to each exponential component, whereas Eq. 1 was extended to

$$I_h(V, t) = [G_{h1}(V, t) + G_{h2}(V, t)] \cdot (V - V_h)$$

Conductance values were derived from amplitude coefficient (A_i) values by applying the relationship: $G_{hi}(V, t) = A_i(V, t)/(V - V_h)$, where the index i is either 1 or 2. The functions describing $m_{\infty i}(V)$ were derived directly from $G_{hi}(V)$ activation curves. The transitions of the activating particle, m_i , were schematized as the following first-order kinetic reaction:



from which it follows

$$m_i(V, t) = m_{\infty i}(V) - [m_{\infty i}(V) - m_{0i}] \cdot \exp(-t/\tau_i) \quad (3)$$

where

$$\tau_i(V) = 1/[\alpha_i(V) + \beta_i(V)] \quad (4)$$

$$m_{\infty i}(V) = \alpha_i(V)/[\alpha_i(V) + \beta_i(V)] \quad (5)$$

Numerical values for the rate constants, α_i and β_i , were derived from the experimental values of time constants of activation and deactivation (τ_i) and from the $m_{\infty i}$ curves by applying Eqs. 4 and 5. Rate-constant plots were best fitted with the empiric function

$$\alpha_i(\text{or } \beta_i) = (a_i \cdot V_m + b_i)/\{1 - \exp[(V_m + b_i/a_i)/k_i]\}$$

After obtaining the analytic functions appropriately describing the voltage dependence of rate constants, the changes in I_h during various simulated current-clamp protocols were numerically reconstructed on the basis of the Eqs. 1 and 2 and Eq. 3 in its differential form.

The basic equations used for describing I_{NaP} were the same as used for I_h (see preceding text, Eqs. 1 and 2). $G_{\text{NaP}}(V)$ was modeled according to the voltage-dependence data reported by Magistretti and Alonso (1999). I_{NaP} activation was assumed to be instantaneous.

Kinetic and voltage-dependence parameters concerning I_h and I_{NaP} were used in a simplified model of an EC SC aimed at reproducing the subthreshold oscillatory behavior of membrane potential in these same neurons. In this model, the neuron was considered as mono-compartmental, and its membrane conductance consisted of G_h , G_{NaP} , and a linear leakage conductance (G_l) whose current reversed at the equilibrium potential for K^+ . The parameters of the equations describing conductance kinetics and voltage dependence were given the same numerical values as returned by the analysis of the relevant experimental data (see RESULTS). Na^+ and K^+ reversal potentials had the theoretical (Nernst) values calculated for the ionic conditions employed in our current-clamp experiments ($V_{\text{Na}} = +87$ mV, $V_{\text{K}} = -83$ mV). The reversal potential for I_h ($V_h = -20$ mV) and the amplitude ratio between the fast and slow kinetic components of G_h ($G_{h1\text{Max}}/G_{h2\text{Max}} = 1.85$) also matched exactly the experimentally observed average values. Only the absolute values of maximal conductances (G_{Max}) were adjusted until a good concordance between simulations and experimental observations was achieved. In the simulations here illustrated, $G_{h\text{Max}}$, G_{NaPMax} , and $G_{l\text{Max}}$ equaled 98.0,

17.4, and 78.0 pS/pF, respectively. These values compared reasonably, namely within a factor of 2, with the experimentally measured values.

Numeric solution of the differential equations was achieved by the use of a one-step Euler integration method. The integration step size was 0.25 ms. Preliminary tests on the adequacy of this integration method were carried out by reducing the step size by ≤ 25 times, which revealed an optimal convergence. The simulation programs were compiled using QuickBASIC 4.5 (Microsoft). Data were analyzed using Origin.

RESULTS

The results presented in this study were based on a database of 131 EC layer II SCs intracellularly recorded under whole cell patch conditions and met the criteria specified in METHODS. The studied neurons were identified as SCs by their gross morphological characteristics (Klink and Alonso 1993) as afforded by direct visualization of their somata and proximal dendrites (Fig. 1A) but mainly by their characteristic electrophysiological properties (Alonso and Klink 1993; Alonso and Llinás 1989) (Fig. 1, B–D). Indeed, as illustrated in Fig. 1, patched SCs demonstrated qualitatively the same electroresponsive properties that distinguish SCs recorded with sharp electrodes. First, the patched SCs demonstrated robust time-dependent inward rectification in the hyperpolarizing direction. As shown in Fig. 1B, the membrane voltage responses to hyperpolarizing current pulses did not monotonically reach a steady value but displayed, after a certain delay, large amplitude “sags” back to more depolarized values. Second, the action potential of the patched SCs also demonstrated the

characteristic fast after hyperpolarization (arrowhead Fig. 1C) followed by a depolarizing afterpotential and a medium after hyperpolarization. Finally, and most importantly, patched SCs also developed rhythmic subthreshold membrane potential oscillations and demonstrated cluster discharge when depolarized with DC current in the membrane potential range between -55 and -50 mV (Fig. 1D, 1–3). At an average membrane potential of -52 ± 1 mV, the peak frequency of these membrane potential oscillations as determined by Fourier analysis averaged 3.1 ± 0.7 Hz ($n = 12$). The SCs had an average resting membrane potential of -55 ± 3 mV and an input resistance of 113 ± 40 M Ω .

Although not further treated, in some instances, neurons other than SCs were recorded from. Pyramidal-like cells of EC layer II ($n = 10$), could be distinguished from SCs based on their pyramidal shape and their limited expression of time-dependent inward rectification (Klink and Alonso 1993). Layer III pyramidal cells ($n = 3$) were distinguishable based on their qualitatively smaller size, their high-input resistance (217 ± 67 M Ω) and the absence of time-dependent inward rectification (Dickson et al. 1997).

Hyperpolarization-activated, time-dependent inward rectification in SCs corresponded to a slow, noninactivating inward current

As illustrated for a typical SC in Fig. 2, the depolarizing sags that developed on membrane hyperpolarization in current-clamp conditions (A, $\leftarrow$) were paralleled by the development

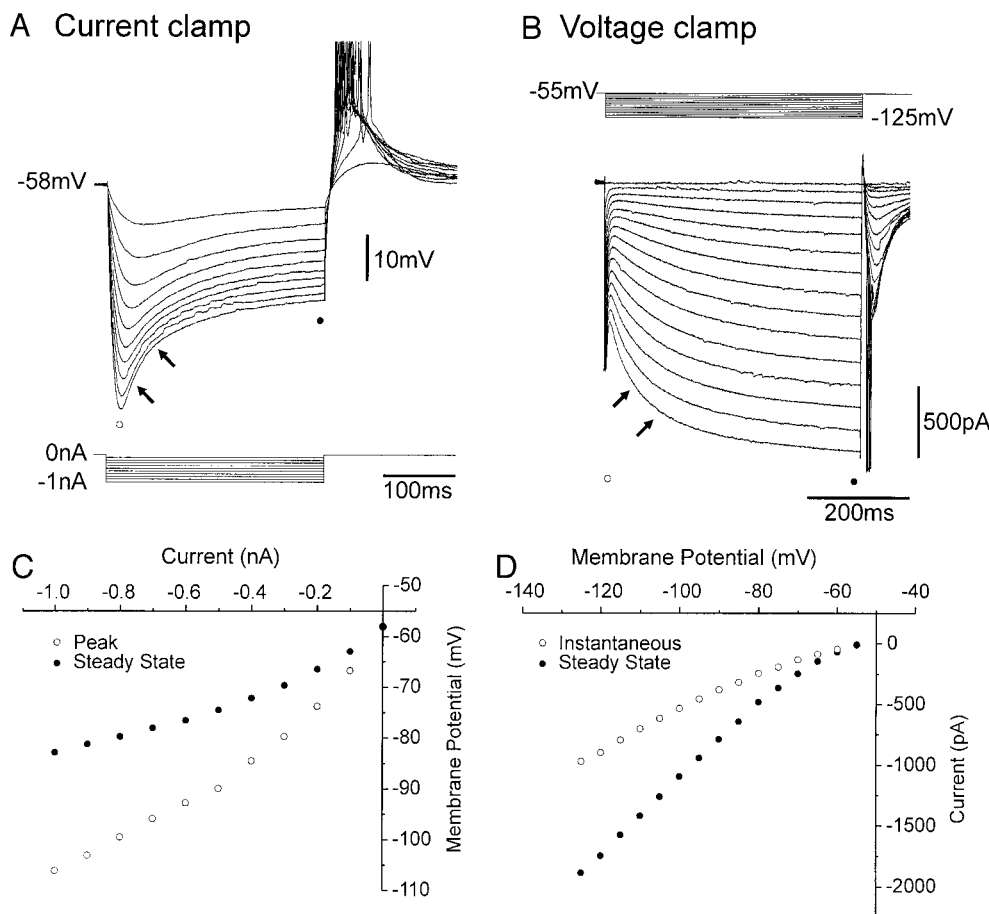


FIG. 2. Characterization of inward rectification in the SCs. A: in current-clamp conditions, hyperpolarizing current pulses evoke voltage responses with a robust time-dependent depolarizing sag ($\leftarrow$). B: in the same neuron, under voltage-clamp conditions, hyperpolarizing voltage steps evoke a slow inward current (I_h) that grows in amplitude and rate of activation with increasing hyperpolarization. C: peak and steady-state V - I plot ($\circ$ and $\bullet$, respectively) derived from the data in A. Time-dependent inward rectification is graphically represented as the depolarizing shift between peak and steady-state potential readings. D: instantaneous and steady-state I - V plot ($\circ$ and $\bullet$, respectively) derived from the data in B. Time-dependent inward rectification is apparent as the negative difference in the holding current between the instantaneous and steady-state measurements. All traces shown were obtained in the standard Ringer solution.

of a slow inward current on step hyperpolarization under voltage-clamp conditions (B, $\rightarrow$). Note that the time course and amplitude of this inward relaxation was overtly voltage dependent (see following text). In all cases, analysis of the subthreshold input-output relations under current-clamp revealed that the steady-state voltage-current (V - I) curve (Fig. 2C; $\bullet$) showed a marked upward bending over the entire voltage range (-60 to -120). Similarly, analysis of input-output relations under voltage-clamp revealed that the steady-state current-voltage curve (ssI - V ; Fig. 2D, $\bullet$) showed a robust inward shift, as compared with the instantaneous current-voltage curve (Fig. 2D, $\circ$), that grew steadily with membrane hyperpolarization. The slow inward current relaxations were associated with a membrane-conductance increase because the instantaneous current flowing at the break of the hyperpolarizing commands was larger than that recorded on first jumping to the command potential (see Fig. 4A). Thus SCs do possess a robust time-dependent hyperpolarization activated conductance (G_h).

Pharmacological block of inward rectification

In addition to a time-dependent inward rectifier such as I_h , many neurons also possess a fast inward rectifier K^+ current (I_{Kir}) (reviewed by Hille 1992). It has been shown that in many cells bath application of Ba^{2+} and Cs^+ can be used to pharmacologically dissect I_h from I_{Kir} because Ba^{2+} blocks I_{Kir} and not I_h , whereas Cs^+ blocks both I_{Kir} and I_h (Hagiwara et al. 1976, 1978). In agreement with this, in all SCs tested ($n = 8$), bath application of Ba^{2+} (0.5 – 2 mM) had no effect on I_h (Fig. 3, A–C), although it did block the small inward bending of the

instantaneous I - V relationship that was always observed at potentials negative to about -80 mV in control conditions (Fig. 3C, squares). This Ba^{2+} effect suggests the presence of a minor I_{Kir} in the SCs. In contrast to Ba^{2+} , in all SCs tested ($n = 10$), bath application of Cs^+ (1 – 6 mM) always produced a substantial decrease in I_h (though never a complete I_h block). This decrease was assessed by expressing the percentage decrease in the difference between the instantaneous and steady-state current at potentials between -60 and -80 mV before and after application of Cs^+ (cf. Ishii et al. 1999). It was dose dependent and ranged from 60 to 75% for a concentration of 2 mM Cs^+ ($n = 5$) that produced close to maximal effects.

Given that Cs^+ produced only a partial block of I_h in the SCs, we assessed the effects of the novel bradycardic agent ZD7288, which has been reported to be a potent blocker of I_h in other cells (BoSmith et al. 1993; Harris and Constanti 1995; Maccaferri and McBain 1996; Williams et al. 1997). As illustrated in Fig. 3 (D and E), in all cases tested ($n = 9$), bath application of ZD7288 (>10 min; 100 μ M) always resulted in a complete and irreversible block of I_h . When the cells were held at -60 mV (about resting level), application of ZD7288 always resulted in an outward shift of the holding current (mean $= 138 \pm 52$ pA, $n = 5$), indicating that G_h is active at the resting membrane potential (see following text). Significantly, ZD7288 did not abolish the small inward shift of the instantaneous I - V relation below -80 mV (Fig. 3G) and thus whereas ZD7288 fully blocked I_h , it did not affect I_{Kir} . Although small, the remaining fast inward rectification, however, could be blocked fully by the further addition of Ba^{2+} that also

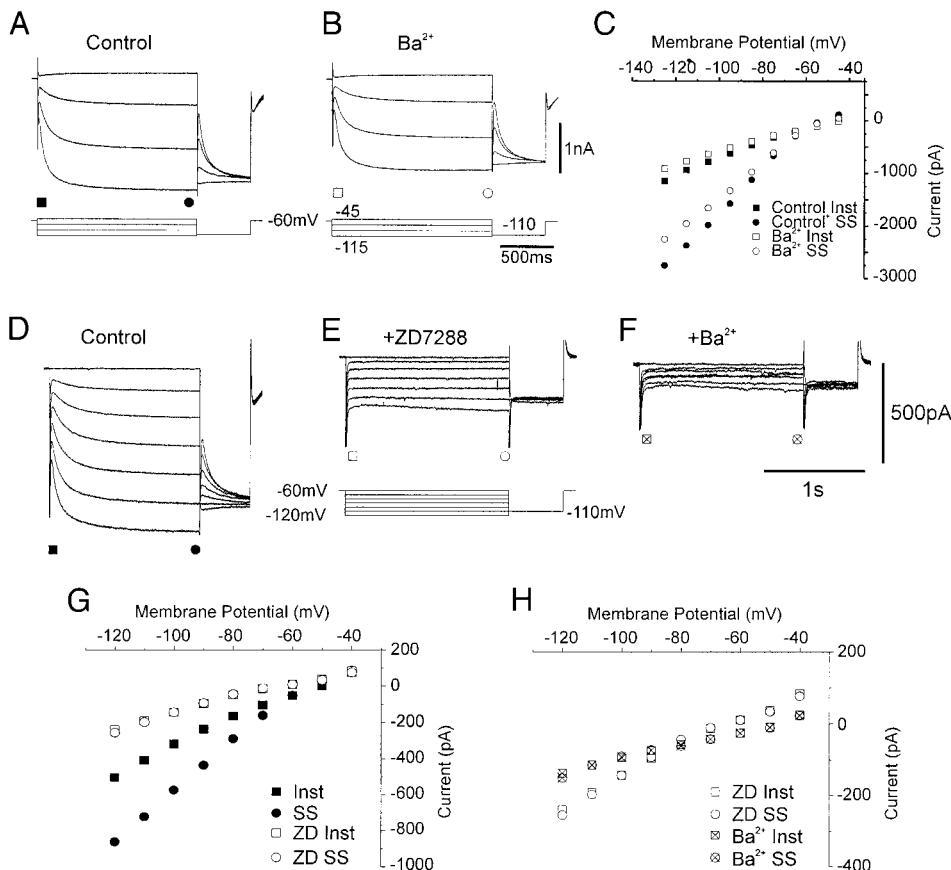


FIG. 3. Effects of extracellular Ba^{2+} and ZD7288 on I_h . A and B: current responses evoked by a series of voltage-clamp steps in control conditions (A) and during superfusion with 2 mM Ba^{2+} (B). C: I - V plot of both the instantaneous ($\blacksquare$ and $\square$) and steady-state ($\bullet$ and $\circ$) current responses from A and B. Note that Ba^{2+} did not block time-dependent inward rectification (generated by I_h) though it did appear to affect fast inward rectification (apparent as the negative bending of the instantaneous current plot at potentials negative to -80 mV). D–F: current responses evoked by a series of voltage-clamp steps in control conditions (D), in the presence of 100 μ M ZD7288 (E) and during further addition of 2 mM Ba^{2+} (F). G: I - V plot of both the instantaneous ($\blacksquare$ and $\square$) and steady-state ($\bullet$ and $\circ$) current responses from (D, control) and (E, +ZD7288). H: I - V plot of both the instantaneous ($\square$ and $\boxtimes$) and steady-state ($\circ$ and $\otimes$) current responses from (E, +ZD7288) and (F, + Ba^{2+}). Note that ZD7288 completely abolished time-dependent inward rectification and that Ba^{2+} abolished the remaining, minor, fast inward rectification. All recordings were carried out in the presence of 1 μ M TTX and 2 mM Co^{2+} .

caused a decrease in slope conductance due to its blocking action on leak currents ($n = 3$; Fig. 3, *F* and *H*).

Activation of I_h

We estimated the activation curve of the membrane conductance underlying I_h (G_h) by applying two different protocols (Fig. 4). In the first protocol, a modified Ringer solution (as specified in METHODS) was used. The activation curve of G_h was estimated from the peak amplitude of the tail currents recorded at about -40 mV ($n = 8$) or at about -60 mV ($n = 5$) after a series of hyperpolarizing voltage-clamp steps from a holding potential in the range of -45 to -30 mV (Fig. 4, *A–C*). When stepping back to -60 mV, the zero current level was the tail current amplitude after the most depolarized voltage step (at least -40 mV). Tail current amplitudes were normalized to the maximal value ($I_{\max}$) and plotted as a function of the membrane potential during the hyperpolarizing prepulse. In all cases ($n = 13$), the data were well fitted with a Boltzmann equation of the form

$$I/I_{\max} = (1 + e^{((V_m - V_{1/2})/k)})^{-1}$$

where V_m is the membrane potential of the prepulse, $V_{1/2}$ the membrane potential at which G_h is half activated, k a slope factor, and I is the amplitude of the tail current recorded after the prepulse. The tail current analysis yielded an activation range of G_h between -45 and -115 mV, a mean value for $V_{1/2}$ of -77 ± 5 mV and a slope factor (k) of 11.2 ± 1.8 (Fig. 4*C*).

For comparison, in four cells we applied slow (<10 mV/s) 100 mV hyperpolarizing voltage ramps from a holding potential of -30 mV in control and after block of I_h with ZD7288 (Fig. 4*D*). In these experiments, $1 \mu\text{M}$ TTX, 2 mM Co^{2+} , and 2 mM Ba^{2+} were added to the control Ringer. Subtraction of the ZD7288 I - V curve from the control curve yielded the steady-state I_h I - V relationship from which we estimated G_h according to the formula $G_h = I_h/(V_m - V_h)$ where V_h is the reversal potential for I_h estimated to be -21 mV (see following text, Fig. 6). The resulting values were normalized to the maximal conductance ($G_{\max}$; 10.4 ± 2.3 nS) and plotted against V_m . In all cases the curves were well fitted with a Boltzmann equation as in the preceding text. This ramp analysis yielded a $V_{1/2}$ of -76 ± 4 mV and a slope factor of 12.1 ± 2 , which were not significantly different to those obtained by tail current analysis by two-tailed t -tests [$t(15) = 0.36$, $P > 0.05$; $t(15) = -0.85$, $P > 0.05$].

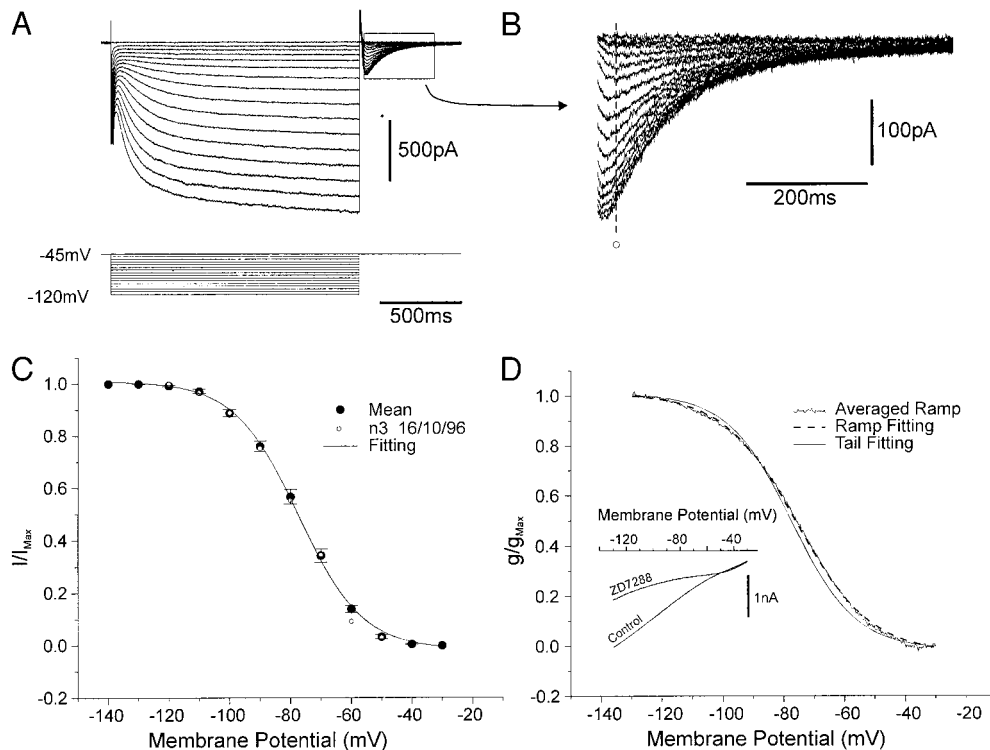


FIG. 4. Activation curve for the conductance G_h . *A*: incremental hyperpolarizing voltage-clamp steps increase the amount of I_h that is activated as well as the amplitude of the tail currents that follow (*inset*). *B*: tail currents from *A* shown at an expanded time and current scale. Tail current amplitude measurements were taken at the time indicated by the empty circle and broken line. *C*: plot of the activation curve of G_h . Filled circles, averaged activation curve for 13 SCs. Individual experiments first were fitted with Boltzmann functions and the interpolated values for the steps at each 10-mV increment were used to compute the average value. Small open circles, data derived from cell in *A*. Line, best Boltzmann fit to the average (see RESULTS) where the half activation voltage and slope factor were found to be -77 ± 5 mV and 11.2 ± 1.8 , respectively. *D*: G_h activation curves also were estimated in four cells from I_h - V relations where I_h was isolated by subtracting ZD7288 I - V curves from control I - V curves obtained through slow ramp protocols (*inset*, see RESULTS for details). As can be seen, the G_h activation curve obtained in this manner (noisy line) and its Boltzmann fit (dotted line) largely overlap the activation curve obtained from tail current analysis (continuous line; same as in *C*). Tail current protocols were conducted using the specialized solution described in METHODS, and ramp protocols were conducted in solution containing $1 \mu\text{M}$ TTX, 2 mM Co^{2+} , and 2 mM Ba^{2+} .

Time course of activation and deactivation

As stated previously, the rate of activation of I_h increased sharply with hyperpolarization (e.g., Fig. 2B). This qualitative observation was further explored in a more quantitative manner. To maximize the accuracy of our kinetic analysis, we isolated I_h by subtracting from control current traces evoked by hyperpolarizing voltage-clamp steps, the current traces evoked to the same potentials in the presence of the selective I_h blocker ZD7288 ($n = 5$; Fig. 5A). Over the whole voltage range tested, the I_h current relaxations were best fitted with a double exponential function of the form

$$I_h(t) = A_1 e^{(-t/\tau_1)} + A_2 e^{(-t/\tau_2)} + C$$

where $I_h(t)$ is the amplitude of the current at time t , C is a constant, and A_1 and A_2 reflect the amplitude coefficients of the fast (τ_1) and slow (τ_2) time constants, respectively. Attempts to fit the subtracted traces with a single exponential function were judged to be unsuccessful based on visual inspection and by comparison of the standard deviations of the fits using single or double exponential functions (not shown). Both the first and second time constants were found to be voltage dependent, as shown in Fig. 5, C and D, becoming faster with increased hyperpolarization. The first time constant ranged between 78 ± 12 and 39 ± 6 ms for voltage steps to -70 and -110 mV, respectively. The second time constant ranged between 372 ± 39 and 164 ± 45 ms for the same voltage steps. The ratio of the amplitude coefficients for the first (A_1) and second (A_2) time constants increased from 1 at -70 mV to just over 2 at -110 mV.

An equivalent method as the one described in the preceding text was conducted to study the rate of deactivation of I_h isolated with the use of ZD7288 (Fig. 5B). Isolated I_h current traces evoked by depolarizing voltage steps from a holding potential of -60 mV were also well fitted by a double-exponential function. As for the time constants of activation, both

the first and second time constants of deactivation were found to be voltage dependent, becoming faster with increasing depolarization (Fig. 5, C and D). The first time constant of deactivation ranged from 23 ± 9 to 58 ± 13 ms for voltage steps to -40 and -50 mV, respectively. The second time constant of deactivation ranged between 241 ± 38 and 326 ± 58 ms for the same voltage steps. The amplitude of the fast time constant was roughly 1.25 that of the slower, and this ratio remained constant over the voltage range tested.

Reversal of I_h

Estimation of the reversal potential of I_h was achieved by two different methods, which took advantage of the fact that at -80 mV, G_h was strongly activated and did not show time-dependent inactivation (Fig. 6, A and B). In all experiments, the superfusing Ringer solution contained $1 \mu\text{M}$ TTX, 2 mM CoCl_2 , and 2 mM BaCl_2 . Thus in the first method, we estimated the reversal potential of I_h (V_h) from the intersection of the instantaneous (chord) current-voltage relationships recorded at holding potentials of -80 and -40 mV (i.e., in the presence and absence of I_h ; Fig. 6C) (Mayer and Westbrook 1983). In 17 neurons examined, this method provided an average value for V_h of $-21 \pm 5 \text{ mV}$.

To support the preceding estimation, we used a second method in which we took advantage of the fact that I_h is selectively and fully blocked by ZD7288 (see preceding text). Chord conductance measurements were made from voltage steps from a holding potential of -80 mV before and after block of I_h using ZD7288 and the instantaneous I/V relationships in both conditions were constructed (Fig. 6, D–F). In eight neurons examined, the average voltage at which the linear fits for both plots intersected, i.e., the reversal potential for I_h , was $-22 \pm 6 \text{ mV}$, a value that was not significantly different from that found with the tail current analysis method above [$t(23) = 0.44$, $P > 0.05$].

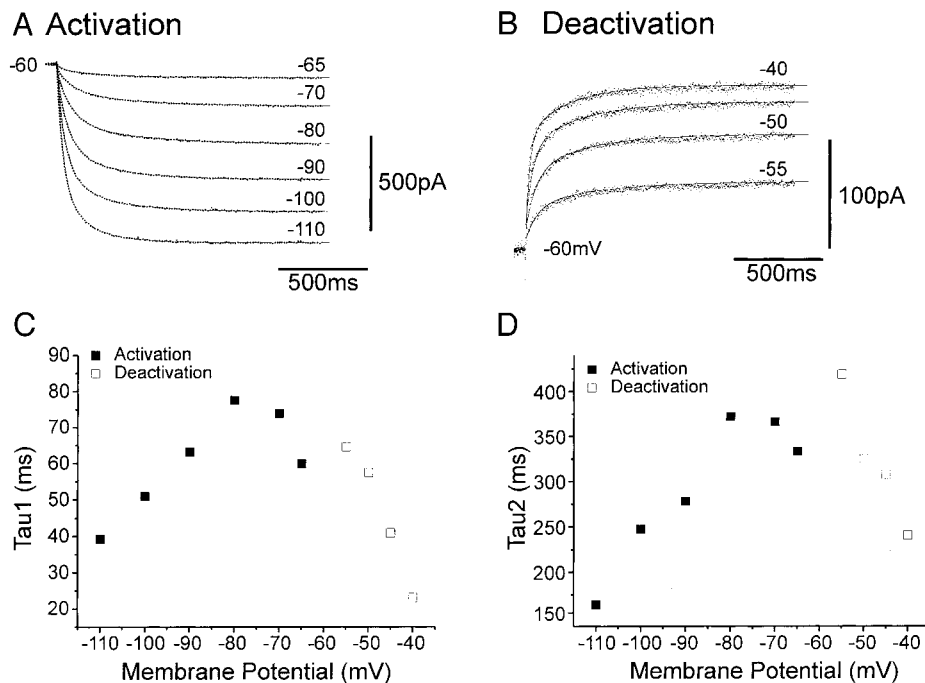


FIG. 5. Activation and deactivation kinetics of I_h . I_h was isolated by subtraction of ZD7288 current traces from control current traces recorded in the presence of $1 \mu\text{M}$ TTX, 2 mM Co^{2+} , and 2 mM Ba^{2+} . A: averaged ($n = 5$) I_h traces ($\cdots$) evoked by hyperpolarizing steps from -60 mV. I_h activation time course was well fitted by a double-exponential decay function (—, see RESULTS for details). B: averaged ($n = 5$) I_h traces ($\cdots$) evoked by depolarizing steps from -60 mV (same collection of cells as in A). I_h deactivation time course was also well fitted by an incremental asymptotic double-exponential function (—, see RESULTS for details). C and D: plots of the 1st and 2nd time constants of activation and deactivation as a function of membrane potential. Kinetics of activation of I_h (■) can be seen to be voltage dependent with both the 1st (C) and 2nd (D) time constants decreasing with hyperpolarization. Kinetics of deactivation of I_h (□) also can be seen to be voltage dependent with both the 1st (C) and 2nd (D) time constants decreasing with depolarization.

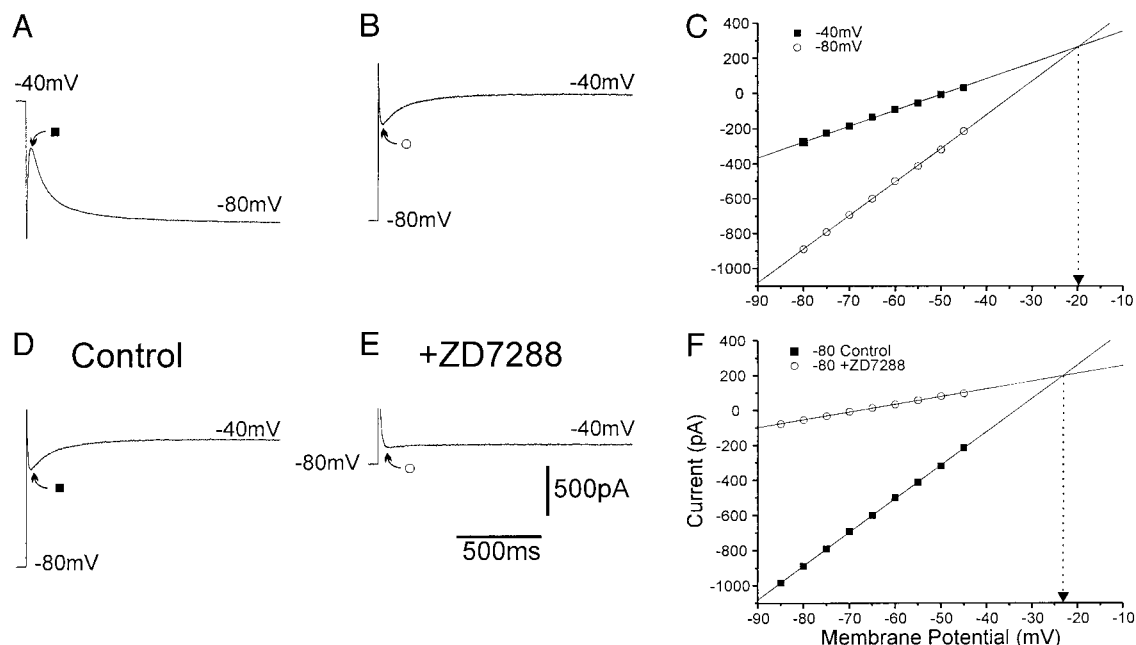


FIG. 6. Reversal potential of I_h . Extrapolated reversal potential of I_h (V_h) was estimated by 2 different approaches. A–C: SCs were held at -40 and -80 mV (A and B, respectively) and the instantaneous $I-V$ relations constructed. V_h was estimated from the intersection of the extrapolated $I-V$ relations derived from both holding potentials (C). D and E: SCs were held at -80 mV, and the instantaneous $I-V$ relation was constructed in control conditions (D) and during I_h block with ZD7288 (E). V_h was estimated from the intersection of the extrapolated $I-V$ relations derived in both conditions (F). All recordings were conducted in a solution containing $1 \mu\text{M}$ TTX, 2 mM Co^{2+} , and 2 mM Ba^{2+} .

Ionic basis of I_h

The fact that in the SCs I_h reverses at about -20 mV suggests that, as in other neurons (Crepel and Penit-Soria 1986; Halliwell and Adams 1982; Mayer and Westbrook 1983; McCormick and Pape 1990; Spain et al. 1987; Takahashi 1990), this hyperpolarization-activated inward current might be carried by a mixture of both Na^+ and K^+ ions. Indeed, increasing the extracellular concentration of K^+ ($[\text{K}^+]_o$) (Fig. 7) produced an increase in I_h (with no change in the G_h activation curve; not shown) as well as an increase in instantaneous conductance. As expected for K^+ being an important carrier for I_h , an increase in $[\text{K}^+]_o$ from 1 to 10 mM produced an average positive shift in V_h of $10 \pm 4 \text{ mV}$ ($n = 4$, Fig. 7D).

On the other hand, reductions in the concentration of extracellular Na^+ from control levels (151 mM) to 26 mM reversibly reduced the amplitude of I_h (Fig. 8) without changing the activation properties of the conductance underlying this current (not shown). Concomitant with this reduction, V_h shifted in the hyperpolarizing direction by an average of $21 \pm 5 \text{ mV}$ ($n = 5$). These results indicate that Na^+ ions also largely contribute to I_h .

Finally, a number of neurons (5) were recorded using a modified intracellular solution containing an additional 10 mM Cl^- in the pipette solution (see METHODS). Although in these cases, the chloride reversal potential was theoretically shifted by ~ 20 mV in a positive direction, no significant difference was observed in either the average reversal potential [$-23 \pm 6 \text{ mV}$; $t(20) = -1.48$, $P > 0.05$] or the activation properties of I_h (not shown). Thus using the Goldman-Hodgkin-Katz equation and an estimated V_h of -21.5 mV , we calculated a permeability (conductance) ratio for Na^+ and K^+ ($p\text{Na}^+/p\text{K}^+$) of ~ 0.4 for I_h in the SCs.

Role of I_h in membrane potential oscillations

Given the overlap between the activation range of G_h (threshold at about -45 mV), and the voltage range at which subthreshold membrane potential oscillations occur in SCs (-60 to -50 mV), we sought to define the involvement of I_h in these oscillations by exploring the effects on them of Cs^+ , ZD7288 and Ba^{2+} . Because these agents, particularly Cs^+ and Ba^{2+} , greatly enhance spontaneous synaptic events, we carried out this analysis during synaptic transmission block with CNQX ($10 \mu\text{M}$), AP5 ($50 \mu\text{M}$), bicuculline ($10 \mu\text{M}$), and 2-OH-saclofen ($100 \mu\text{M}$). In line with a role of I_h in the generation of the rhythmic subthreshold oscillations, we observed that the addition of Cs^+ ($1\text{--}2 \text{ mM}$; $n = 4$) to the superfusate resulted in a progressive disruption (and slow-down) of the oscillations. However, as previously reported (Klink and Alonso 1993), some trains of subthreshold oscillatory activity could consistently be observed in the presence of Cs^+ . This result might be interpreted as suggestive that, in addition to I_h , another conductance operating in the subthreshold range, such as the M current, may play a major role in the generation of the rhythmic subthreshold oscillations by the SCs. Alternatively, it also might be that the expression of subthreshold oscillatory activity by the SCs is rather insensitive to the level of I_h expression and that a major decrease in I_h is necessary to abolish the oscillations. To explore these possibilities, we first tested the effects of the more potent I_h blocker ZD7288. In all cells, application of $50\text{--}100 \mu\text{M}$ ZD7288 always resulted in membrane hyperpolarization ($9 \pm 4 \text{ mV}$; $n = 8$) concomitant with the block of the typical depolarizing voltage sag evoked by hyperpolarizing current pulses. Similarly to Cs^+ , ZD7288 always produced a progressive disruption of the oscillations, though, in contrast to what was ob-

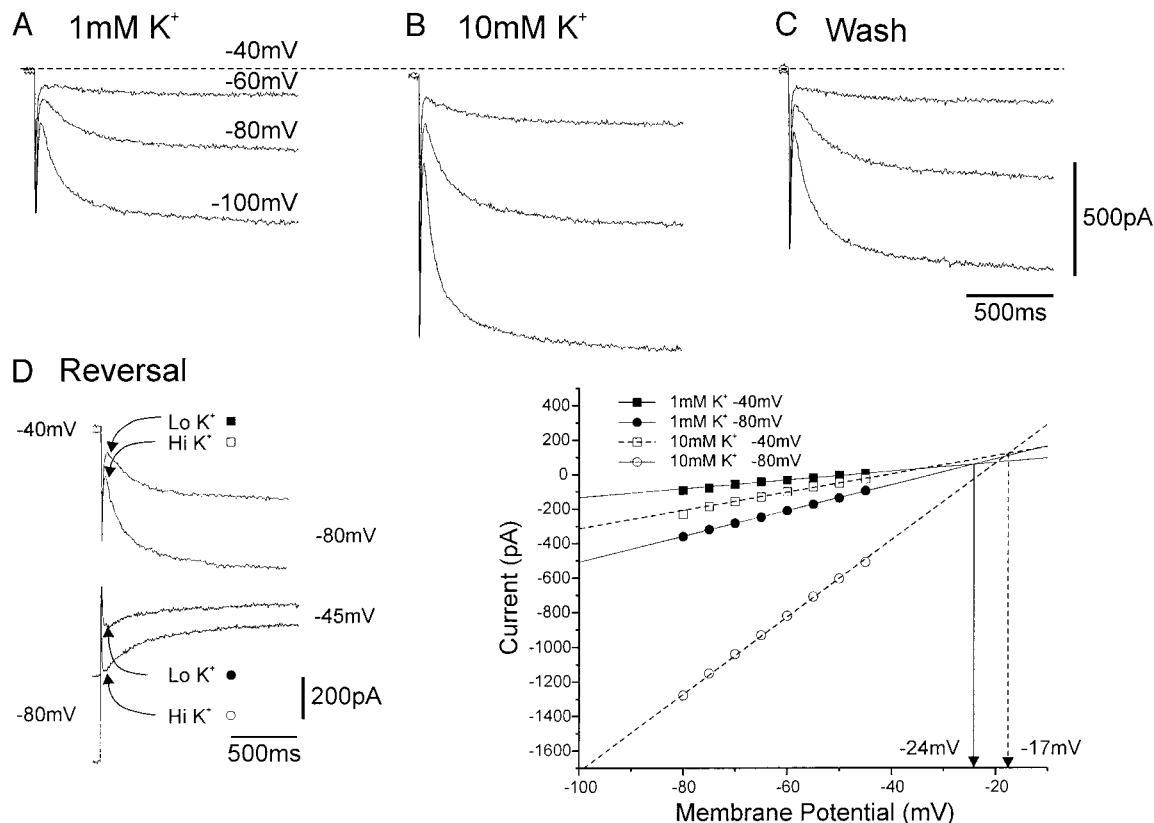


FIG. 7. I_h is increased and V_h shifted in a depolarized direction in raised $[K^+]_o$. A: I_h was evoked by hyperpolarizing voltage steps from a holding potential of -40 mV in a control Ringer solution containing 1 mM $[K^+]_o$. B: increasing the concentration of $[K^+]_o$ markedly increased the amplitude of I_h evoked at the same potential levels. C: washing with 1 mM $[K^+]_o$ reversed this increase. D: cells were held at -40 and -80 mV (top and bottom left, respectively) and the instantaneous I -V relations were constructed in both 1 and 10 mM $[K^+]_o$. Right: V_h in both 1 mM ($\bullet$, $\blacksquare$, and $—$) and 10 mM $[K^+]_o$ ($\circ$, $\square$, and $- - -$) was estimated from the intersection of the extrapolated I /V relations constructed from -40 ($\square$ and $\blacksquare$) and -80 mV ($\bullet$ and $\circ$). Note that in this cell changing the $[K^+]_o$ from 1 to 10 mM shifted the extrapolated V_h from -24 to -17 mV. All recordings were conducted in a solution containing 1 μ M TTX, 2 mM Co^{2+} , and 2 mM Ba^{2+} .

served with Cs^+ , this disruption always proceeded to a complete block (Fig. 9, A–C). Although these data suggest that, indeed, a major block of I_h is necessary to completely abolish the oscillations, it could be argued that the blocking effect of ZD7288 might have been due to a nonselective action of the drug on another conductance operating in the oscillatory range. To exclude this possibility, we performed a series of voltage-clamp experiments in which we examined the effects of ZD7288 on the outward current relaxations evoked by a series of depolarizing voltage-clamp steps from -60 mV (about resting level) to the voltage range where subthreshold oscillations develop (-55 to -50 mV; Fig. 10A) and up to the G_h activation threshold (-45 mV). These experiments were conducted in the presence of 1 μ M TTX and 2 mM Co^{2+} . As shown in Fig. 10, B–D, ZD7288 (100 μ M) always caused a robust outward shift in the holding current and a complete and selective block of both the outward current relaxations in response to membrane depolarization as well as the associated tail currents on return to the holding potential ($n = 4$). In contrast, there was a nearly perfect overlap between the traces at -45 mV, the threshold for activation of I_h , before and after ZD7288. This indicates that, in the voltage range from -60 to -45 (which includes the voltage range at which the membrane potential oscillations occur) the action of ZD7288 was specific

for I_h . Thus the block of the oscillations by ZD7288 cannot be attributed to a nonspecific effect of the drug.

Finally, it also might be argued that the disappearance of sustained subthreshold oscillations with ZD7288 resulted from the membrane conductance decrease due to the I_h block and not by the I_h block per se. This possibility was tested with the use of Ba^{2+} (1 – 2 mM; $n = 7$), which, in contrast to ZD7288, does not affect I_h (cf. Fig. 3) but which, similarly to ZD7288, also produces a major decrease in membrane conductance. Importantly, and in sharp contrast to the ZD7288 results, bath superfusion with Ba^{2+} resulted in both a significant increase in the amplitude [1.2 ± 0.5 mV; $t(4) = 2.7$; $P < 0.05$] and a significant decrease in the frequency [-1.3 ± 0.1 Hz; $t(6) = 8.8$; $P < 0.01$] of the subthreshold oscillations (Fig. 9, D–F). In consequence, the above indicates that I_h plays an essential role in the generation of rhythmic subthreshold oscillations by the SCs and that leak conductances can modulate their amplitude and frequency through their effects on passive membrane properties.

Role of I_h and I_{NaP} in the generation of subthreshold oscillations

Although the preceding experimental data indicate that I_h is necessary for the genesis of subthreshold oscillations by the

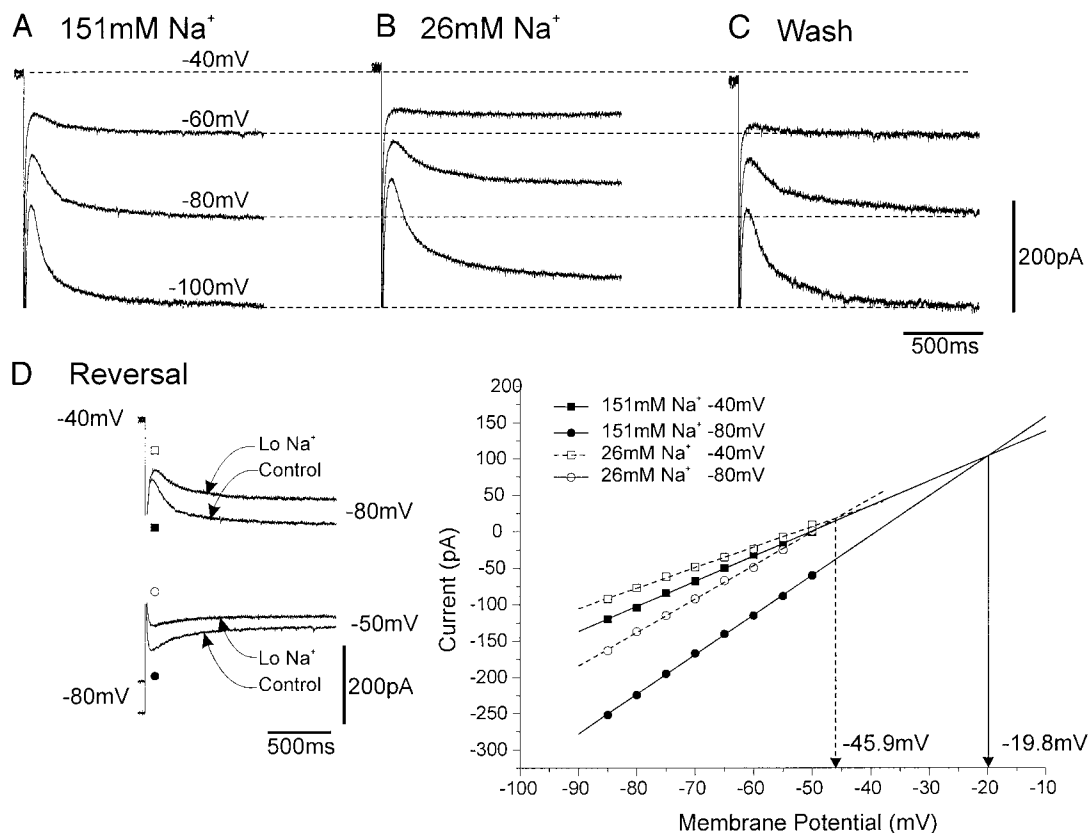


FIG. 8. I_h is decreased and its reversal potential shifted in a hyperpolarized direction in lowered $[Na^+]_o$. A: I_h was evoked by hyperpolarizing voltage steps from a holding potential of -40 mV in a control Ringer solution (151 mM $[Na^+]_o$). B: lowering $[Na^+]_o$ to 26 mM (substitution with *N*-methyl-D-glucamine) diminished the amplitude of I_h evoked at the same potential levels. C: washing with control Ringer reversed this reduction. D: cells were held at -40 and -80 mV (top and bottom left, respectively) and the instantaneous I -V relations constructed in both 151 mM $[Na^+]_o$ (control) and 26 mM $[Na^+]_o$ (low Na^+). Right: V_h in both 151 mM $[Na^+]_o$ ($\blacksquare$, $\bullet$, and $—$) and 26 mM $[Na^+]_o$ ($\square$, $\circ$, and $- - -$) was estimated from the intersection of the extrapolated I -V relations constructed from -40 ($\square$ and $\blacksquare$) and -80 mV ($\circ$ and $\bullet$). Note that in this cell changing the $[Na^+]_o$ from 151 to 26 mM shifted the extrapolated V_h from -19.8 to -45.9 mV. All recordings were conducted in a solution containing 1 μ M TTX, 2 mM Co^{2+} , and 2 mM Ba^{2+} .

SCs, previous studies have shown that these oscillations are also dependent on the activation of a subthreshold persistent Na^+ current (I_{NaP}) (Alonso and Llinás 1989). To generate an oscillatory phenomenon, a process is needed the action of which feeds-back to slow down the rate of the process itself and, most critically, a delay in the execution of the feedback. In SCs, the slow kinetics of activation and deactivation of I_h potentially can implement such a feedback process. To further clarify the role of I_h in the generation of subthreshold oscillations by the SCs and to complement the preceding experimental data, we next implemented a simplified biophysical simulation of the subthreshold membrane voltage behavior of these neurons. Using classical Hodgkin-Huxley formalism, a theoretical reconstruction of the biophysical properties of I_h first was carried out. To be consistent with our experimental data indicating both a fast and a slow kinetic component of I_h (see preceding text; Fig. 5), we constructed activation plots of the corresponding fast and slow conductance components (G_{h1} and G_{h2} , respectively; Fig. 11A, 1 and 2) from which the m_{∞} curves were derived directly by using a standard Boltzmann fitting (see METHODS and legend of Fig. 11). The voltage dependence of the fast and slow rate constants (α_i and β_i ; Fig. 11C, 1 and 2) was estimated from the corresponding time constants of

activation and deactivation (τ_i ; Fig. 11B, 1 and 2) and the m_{∞} curves, as explained in detail in METHODS.

The derived parameters for the kinetics and voltage dependence of I_h and those for I_{NaP} as described previously (Magistretti and Alonso 1999) then were incorporated in a single compartment model of the SC (see METHODS). The model then was explored to test whether it could reproduce characteristic current-clamp phenomena such as the sag in membrane potential during hyperpolarizing current steps and the generation of subthreshold membrane potential oscillations.

Voltage responses to hyperpolarizing current steps in the model SC are illustrated in Fig. 12A. Note that the model cell did display the typical delayed large-amplitude depolarizing sags in response to membrane hyperpolarization as well as robust rebound potentials at the break of the hyperpolarizing current pulses. More importantly, as shown in Fig. 12B, the model SC also developed sustained rhythmic membrane potential oscillations in response to DC membrane depolarization from its resting level to about -53 mV. The combined experimental and model work thus demonstrates that in the SCs the interplay between I_h and I_{NaP} is essential for the generation of sustained rhythmic subthreshold membrane potential oscillations.

As illustrated in Fig. 12C, the use of the model SC also

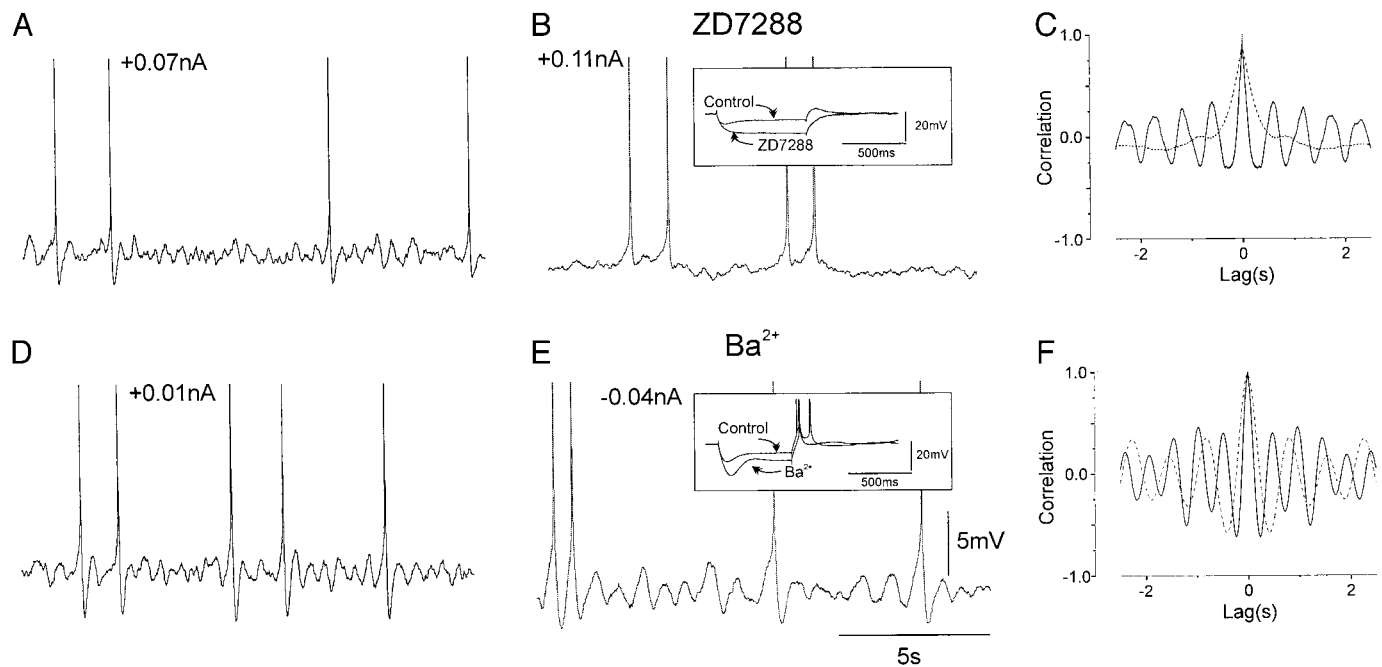


FIG. 9. Effects of ZD7288 and Ba^{2+} on subthreshold oscillations. *A* and *D*: in control Ringer solution containing synaptic blockers (see METHODS), DC depolarization elicited rhythmic subthreshold membrane potential oscillations in 2 different cells. *B*: in the cell shown in *A*, the subthreshold oscillatory activity was abolished after application of $50 \mu\text{M}$ ZD7288. *Inset* in *B* shows the membrane responses to a 0.1-nA hyperpolarizing pulse before and after ZD7288 application, demonstrating a full block of time-dependent inward rectification. *C*: autocorrelation analysis of membrane potential in the just-subthreshold range before and after application of ZD7288 (— and ---, respectively). Note lack of rhythmic activity after drug application. *E*: in the cell shown in *D*, the oscillations were still present after application of 1 mM Ba^{2+} ; however, they were increased markedly in amplitude (from 3.6 to 4.5 mV : peak to peak), and reduced in frequency (from 2.1 to 1.1 Hz). *Inset* in *D* shows the membrane response to a hyperpolarizing pulse of 0.1-nA demonstrating a large increase in input resistance without block of time-dependent inward rectification. *F*: autocorrelation analysis of membrane potential in the just-subthreshold range before and after application of Ba^{2+} (— and ---, respectively). Note the longer period of rhythmic oscillatory activity after Ba^{2+} application.

allowed us to understand the dynamics of the interplay between I_{NaP} and I_h (as well as G_{NaP} and G_h) during the oscillatory cycle. Note that at the trough of an oscillation (1st vertical dashed line) I_{NaP} and G_{NaP} are at a minimum, whereas I_h and G_h are approaching, but not yet at, their respective maxima. This occurs after a certain delay (G_h maximum lags G_{NaP} and V_m minimum by 21 ms at 3 Hz), and thus the attainment of a maximum level by I_h coincides with the initiation of a depolarizing phase. As depolarization proceeds, I_{NaP} rapidly increases because G_{NaP} becomes progressively activated. In turn, the depolarization boosted by I_{NaP} leads to G_h deactivation and thus a decrease in I_h , which slows down and eventually stops the depolarization. Note that at the peak of the oscillation (2nd dashed line) I_h and G_h (similarly to what reciprocally occurs at the trough) are approaching, but they are still not at, a minimum. This occurs after the peak. Thus the deactivation of G_h is now responsible for initiating the repolarizing phase of the oscillation. Membrane hyperpolarization leads to a rapid decrease in I_{NaP} that boosts further hyperpolarization. Eventually membrane hyperpolarization leads to the new activation of I_h and a new oscillatory cycle is initiated.

It can be observed from the traces in Fig. 12C that I_{NaP} essentially changes instantaneously with changes in V_m , whereas changes in I_h follow with a certain delay (caused by its time-dependent properties). This differential behavior may be better understood by plotting I_{NaP} and I_h as a function of V_m as illustrated in Fig. 13. Note that although the I_h curve demonstrates substantial hysteresis, the I_{NaP} curve demonstrates very

little to none. The increase and decrease in I_{NaP} during the depolarizing and hyperpolarizing phase of an oscillation maintains an almost perfect linear relationship with changes in V_m . In contrast, the trajectory followed by I_h during the depolarizing phase of the oscillation is substantially different from that followed during the hyperpolarizing phase. I_h decreases slowly during the initial part of the depolarizing phase but this rate of decrease accelerates as the membrane potential approaches its peak value. Similarly, during the hyperpolarizing phase of the oscillation I_h increases slowly during the initial portion, and this rate of increase accelerates as the membrane potential approaches its minimal value. Basically, the hysteresis introduced by the kinetic properties of I_h implement a delayed feedback mechanism to the voltage changes led by I_{NaP} that allows sustained oscillatory activity to occur.

DISCUSSION

The present results demonstrate that the robust hyperpolarization-activated time-dependent inward rectification displayed by the stellate cells from EC layer II is due to an inward current that we identified as I_h on the basis of its pharmacological and biophysical profile as described in other excitable cells (see recent revisions by Clapham 1998; Pape 1996). Importantly, our study also points out that some of the specific biophysical properties of I_h in the stellate cells determine that this current plays a major “pacemaker” role in the generation of the theta-like subthreshold oscillations typical of these neurons (Alonso

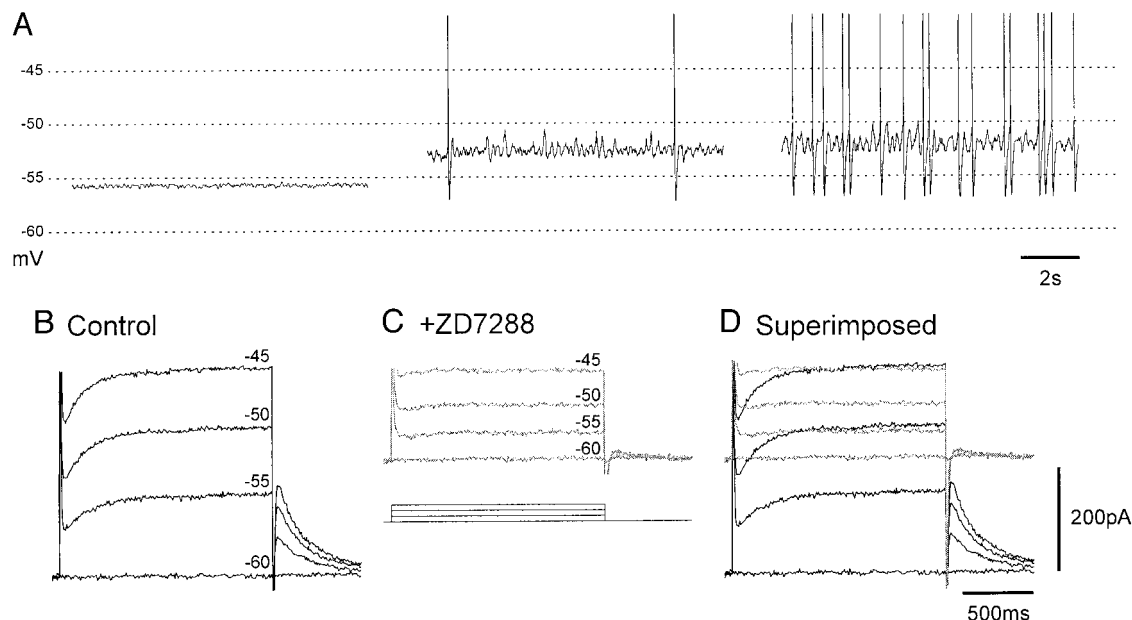


FIG. 10. Blocking effect of ZD7288 on the subthreshold membrane potential oscillations can be attributed to the blocking action of this drug on I_h . *A*: subthreshold oscillations in the SCs develop on DC depolarization to the voltage range between about -55 to -50 mV. *B*: voltage-clamp experiment in the same cell showing outward current relaxations (corresponding to I_h deactivation) during depolarizing voltage steps through the oscillatory voltage level (-55 , -50 , and -45 mV), as well as inward current relaxations (corresponding to I_h activation) on return to the holding potential (-60 mV). *C*: addition of $100 \mu\text{M}$ ZD7288 eliminated I_h and thus caused a robust outward shift in the holding current and the concomitant disappearance of the outward current relaxations on depolarization as well as the inward current relaxations on return to the holding potential. *D*: superimposition of traces in *B* and *C*. Note that control and ZD7288 current traces to -45 mV overlap perfectly at steady-state, thus indicating that in the voltage range examined the actions of ZD7288 were selective for I_h . Voltage-clamp recordings were performed in the presence of $1 \mu\text{M}$ TTX and 2 mM Co^{2+} .

and Llinás 1989). Indeed, the combined experimental and modeling analysis we carried out indicate that the kinetic properties of both the activation and deactivation of I_h implement a delayed feed-back mechanism to the voltage changes led by a subthreshold Na^+ current that allows persistent subthreshold oscillatory activity to occur. Although h currents have been shown to contribute to rebound activity in many neuronal types (Crepel and Penit-Soria 1986; Mayer and Westbrook 1983; Spain et al. 1987) and to interact with low-threshold Ca^{2+} currents (at rather negative levels; about -70 mV) to generate oscillatory activity (Bal and McCormick 1997; Brown and DiFrancesco 1980; McCormick and Pape 1990), to our knowledge, the SCs are the first case in which an h current has been shown to generate persistent oscillatory activity by interacting with a sustained Na^+ current in the subthreshold voltage range (about -55 mV). Intrinsic oscillatory activity in the subthreshold voltage range may be of fundamental importance in defining the integrative properties of the participating neurons (cf. Hopfield 1995; Lampl and Yarom 1993; Llinás 1988).

A characteristic pharmacological feature of I_h in all other cell types studied is its blockade by Cs^+ but not Ba^{2+} . Consistent with this, I_h in the SCs was largely reduced, though not completely blocked, by Cs^+ ($1\text{--}6 \text{ mM}$) and not significantly affected by Ba^{2+} ($1\text{--}2 \text{ mM}$). The incomplete block of I_h by Cs^+ , which also has been observed in other cells (Champigny and Lenfant 1986; Ishii et al. 1999), prompted us to use the recently described bradycardic agent ZD7288 (BoSmith et al. 1993). As found in other neurons (Gasparini and DiFrancesco 1997; Harris and Constanti 1995; Lüthi et al. 1998; Maccaferri

and McBain 1996), ZD7288 fully and selectively (in the voltage range explored: -45 to -120 mV) blocked I_h in the SCs.

In general terms, the biophysical properties of I_h as recorded in the SCs were similar to those reported for other brain neurons though some differences in activation threshold and reversal potential were apparent, and these appear to be functionally significant. I_h in the SCs appeared as a noninactivating inward current that turned on (activated) relatively slowly with hyperpolarization and also turned off (deactivated) slowly with depolarization. As with other voltage-dependent currents, the conductance that gives rise to I_h showed a sigmoidal activation curve with a threshold at around -45 mV, a half-activation point at -77 mV, and a slope factor of 12.1. A half-activation point in the range of -70 to -80 mV is typical of other neurons; however, the I_h activation threshold that we observed in the SCs was $\sim 10\text{--}15$ mV more positive than that reported for other subcortical or cortical neurons (Bayliss et al. 1994; Crepel and Penit-Soria 1986; Halliwell and Adams 1982; Kamondi and Reiner 1991; Mayer and Westbrook 1983; McCormick and Pape 1990; Mercuri et al. 1995; Spain et al. 1987). An activation threshold at about -45 mV is, however, comparable with that found in cardiac sinoatrial cells and Purkinje fibers (DiFrancesco 1981; DiFrancesco et al. 1986; Yanagihara and Irisawa 1980) and to recent findings for hippocampal and brain stem neurons (Maccaferri and McBain 1996; Maccaferri et al. 1993; Travagli and Gillis 1994). In addition, the estimated reversal potential for I_h in the SCs (about -20 mV) was $10\text{--}30$ mV more positive than that reported for other subcortical or cortical neurons (Bayliss et al. 1994; Crepel and Penit-Soria 1986; Halliwell and Adams 1982; Kamondi and Reiner

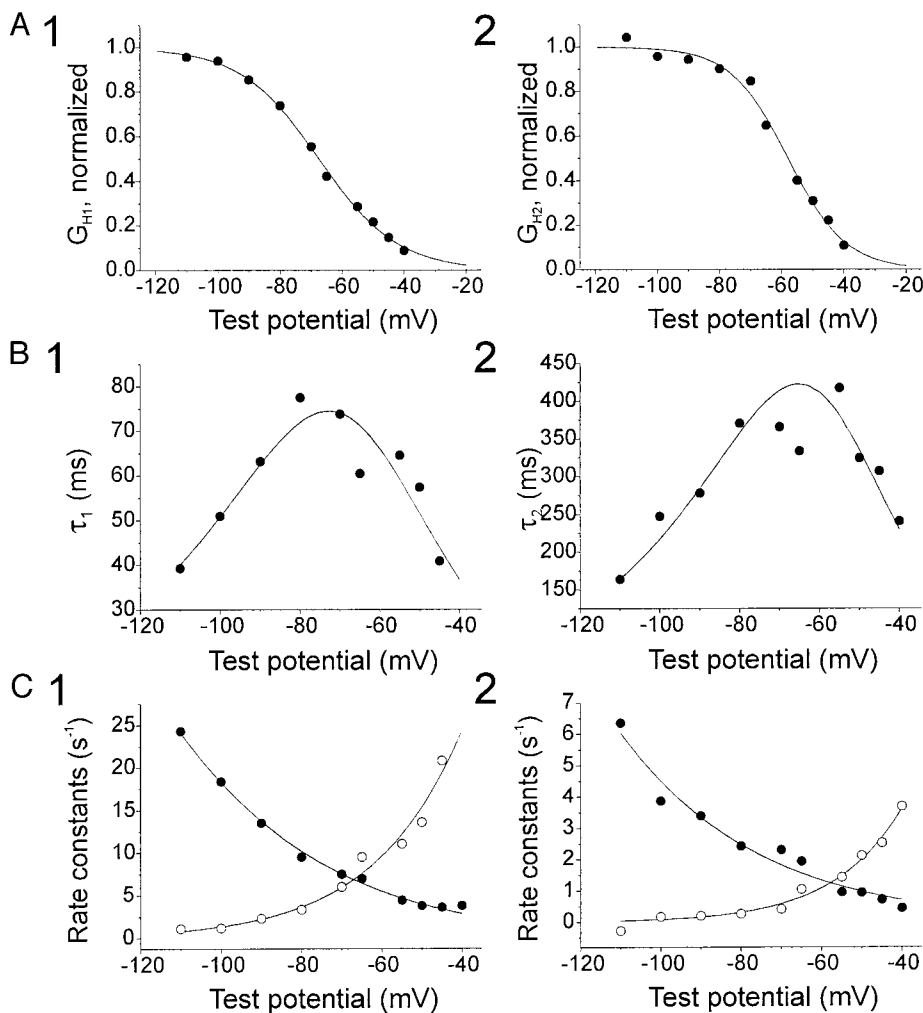


FIG. 11. Modeling of the biophysical properties of I_h . A: voltage dependence of the normalized conductances derived from amplitude coefficients returned by double-exponential fittings of average I_h traces. Amplitude coefficients relating to the fast and slow time constants (A, 1 and 2, respectively) were used to calculate the values of the underlying conductances (G_{A1} and G_{A2} , respectively) as explained in METHODS. Boltzmann fittings to the experimental data points also are shown. Fitting parameters are: $V_{1/21} = -67.4$ mV, $k_1 = 12.66$ mV (A1); $V_{1/22} = -57.92$ mV, $k_2 = 9.26$ mV (A2). B: voltage dependence of fast (1) and slow (2) time constants (τ_1 and τ_2 , respectively) returned by double-exponential fittings of average I_h traces. —, theoretical functions describing the behavior of the experimental plots, as obtained from the theoretical functions derived from fittings of rate-constant plots (see following text; see also METHODS for details). C: voltage dependence of fast and slow rate constants (α_1 , β_1 , and α_2 , β_2 , respectively) calculated from normalized conductance values and time-constant values as explained in METHODS. ●, “on” reaction rate constants (α); ○, “off”-reaction rate constants (β). —, fittings obtained using the theoretical function described in METHODS. Fitting parameters are: $a = -2.89 \cdot 10^{-3}$ mV $^{-1} \cdot$ ms $^{-1}$, $b = -0.445$ ms $^{-1}$, $k = 24.02$ mV (α_1); $a = 2.71 \cdot 10^{-2}$ mV $^{-1} \cdot$ ms $^{-1}$, $b = -1.024$ ms $^{-1}$, $k = -17.4$ mV (β_1); $a = -3.18 \cdot 10^{-3}$ mV $^{-1} \cdot$ ms $^{-1}$, $b = -0.695$ ms $^{-1}$, $k = 26.72$ mV (α_2); $a = 2.16 \cdot 10^{-2}$ mV $^{-1} \cdot$ ms $^{-1}$, $b = -1.065$ ms $^{-1}$, $k = -14.25$ mV (β_2).

1991; Mayer and Westbrook 1983; McCormick and Pape 1990; Mercuri et al. 1995; Spain et al. 1987), though, again, very similar to the value reported for cardiac sinoatrial cells and Purkinje fibers (around -25 mV) (DiFrancesco 1981; DiFrancesco et al. 1986; Yanagihara and Irisawa 1980). A reversal potential around -20 mV clearly suggests that I_h in the SCs, as in all other excitable cells studied, is a mixed cationic current carried by both Na^+ and K^+ ions. Consistent with this interpretation, we found that raising $[\text{K}^+]_o$ shifted V_h in the depolarizing direction (without any significant effect on the activation curve) (Halliwell and Adams 1982; Mayer and Westbrook 1983; Spain et al. 1987; Takahashi 1990), whereas lowering $[\text{Na}^+]_o$ shifted V_h in the hyperpolarizing direction. Alterations in $[\text{Cl}^-]_i$ changed neither the reversal potential nor the activation curve corresponding to this current, suggesting that it is cation-specific.

With regard to the time-dependent properties of I_h in the SCs, an interesting feature was their biexponential kinetic nature. We found that the time course of activation of I_h was described best by a biexponential function having fast (τ_1) and slow (τ_2) time constants that were voltage dependent—decreasing with increasing hyperpolarization. A dual exponential nature of the I_h deactivation time course also was confirmed, with fast and slow time constants that also showed voltage dependency—becoming faster with increasing depolarization.

Although many of the studies on I_h report a time course of activation that appears to be well fitted by a mono-exponential function (Crepel and Penit-Soria 1986; DiFrancesco et al. 1986; Halliwell and Adams 1982; McCormick and Pape 1990), some also have shown that a biexponential fitting best represented the time course of I_h in the corresponding cells (Mayer and Westbrook 1983; Solomon and Nerbonne 1993; Spain et al. 1987). The fact that the activation time constants reported in different neurons (measured at similar temperatures) range from tens of milliseconds (Crepel and Penit-Soria 1986), through hundreds of milliseconds (Kamondi and Reiner 1991) and even seconds (McCormick and Pape 1990; Soltesz et al. 1991), might be interpreted as indicative of multiple I_h channel subtypes with different gating properties. Thus cells expressing more than one I_h channel subtype would show multiple kinetic components during both the activation and deactivation processes. Strong evidence for this comes from a zebrafish mutant (*slow mo*), which exhibits a slower than normal heart rate. A voltage-clamp analysis in isolated cardiac myocytes from the wild-type and the mutant zebrafish demonstrated that I_h was selectively decreased in the mutant. Importantly, this decrease appeared to result from the selective diminution of the fast kinetic component of the current (Baker et al. 1997). Finally, very recently, three different groups have cloned various genes responsible for I_h clearly demonstrating the existence of a

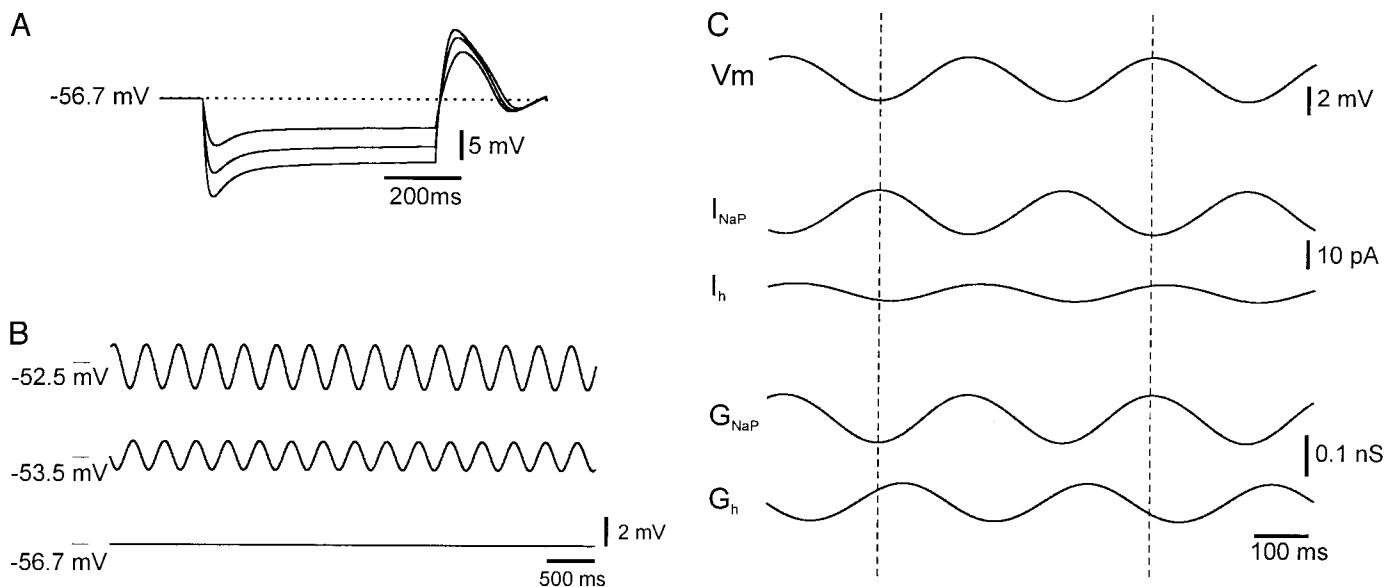


FIG. 12. Modeling of the current-clamp behavior of SCs. A: current-clamp V-I simulation in a simplified model of a SC. In this simulation, 600-ms hyperpolarizing current pulses of -0.49 , -0.98 , and -1.46 pA/pF were injected into the cell from the resting membrane potential level (-56.7 mV). Simulation parameters were: $G_{hMax} = 98.0$ pS/pF, $G_{h1Max}/G_{h2Max} = 1.85$, $G_{NaPMax} = 17.4$ pS/pF, $G_{iMax} = 78.0$ pS/pF, $V_h = -20$ mV, $V_{Na} = +87$ mV, $V_i = -83$ mV. B: simulations of I_h - and I_{NaP} -dependent subthreshold membrane-voltage (V_m) oscillations. Oscillations were elicited by injection of steady depolarizing current of variable amplitude (from bottom to top: 0.00, +0.19, and +0.21 pA/pF). Note the absence of oscillations at rest (-56.7 mV) and the gradual increase in the amplitude of the oscillations with increasing current amplitude. Simulation parameters were the same as described for A. C: expanded view of the simulated membrane potential oscillations evoked by the injection of +0.21 pA/pF and of the behavior of the underlying currents (I_h , I_{NaP}) and conductances (G_h , G_{NaP}). Vertical, dashed lines help in the appraisal of the phase relationships between the membrane potential, the currents, and their conductances. Note the almost perfect phase correspondence between V_m and I_{NaP} , although there appears to be a phase lag between V_m and I_h .

family of hyperpolarization-activated cation channels with different activation kinetics (Gauss et al. 1998; Ludwig et al. 1998, 1999; Santoro et al. 1998).

Function of I_h in the stellate cells

In the stellate cells, the rather positive values that we found for I_h activation (-45 mV) and reversal potential (-20 mV) indicate that in these neurons I_h must be a major contributor to the resting membrane potential (around -60 mV) (Alonso and

Klink 1993). Indeed, full block of I_h with ZD7288 induced a robust hyperpolarization of the resting membrane potential in current-clamp conditions and a large outward shift in the holding current at -60 mV in voltage-clamp conditions. In addition, in the stellate cells, I_h also efficiently modulates membrane hyperpolarization, as observed by the pronounced depolarizing sags in response to membrane hyperpolarization and the robust rebound potentials that follow. In this respect, the stellate cells are densely innervated (Finch et al. 1988; Jones 1994; Jones and Buhl 1993; Kohler 1988; Kohler et al.

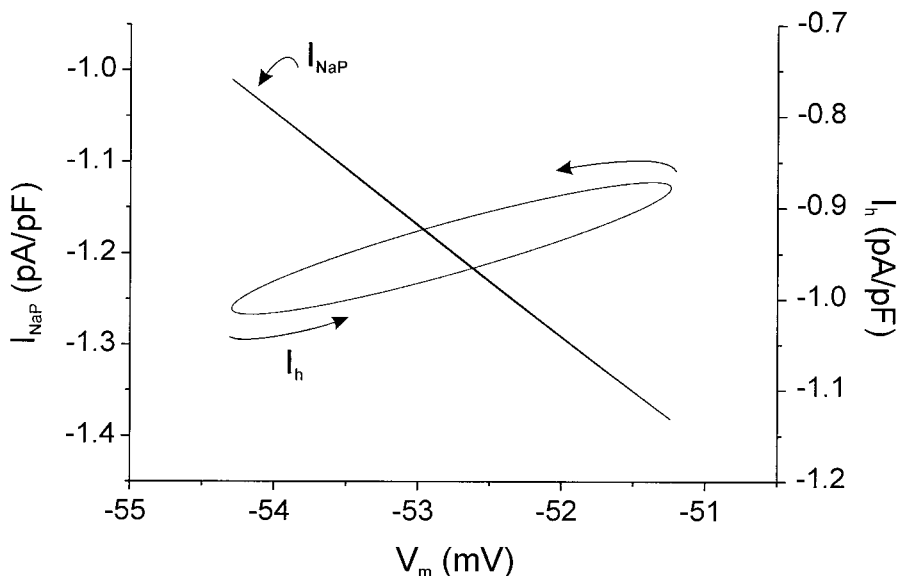


FIG. 13. Phase plane plot of the simulated membrane potential vs. the amplitude of I_{NaP} and I_h . For the simulation shown in Fig. 12C, the amplitude of both I_{NaP} and I_h were plotted with respect to the membrane potential. Plot corresponding to I_{NaP} travels a straight line with negative slope showing essentially no hysteresis. Therefore during both the upswing and downswing of the oscillation there is a relatively "instantaneous" increase and decrease, respectively, in the amplitude of I_{NaP} . On the other hand, the plot corresponding to I_h demonstrates a large degree of hysteresis. Therefore oppositely to I_{NaP} , during the upswing and downswing of the oscillation there is a delayed decrease and increase, respectively, in the amplitude of I_h . Because of this delay, the maxima and minima of the amplitude of I_h tend to occur just after the membrane potential has achieved its most hyperpolarized and depolarized values, respectively (i.e., just after the commencement of the upswing and downswing of the oscillation, respectively).

1985; Wouterlood et al. 1995) by GABAergic inputs, and we have observed that I_h efficiently limits the inhibitory postsynaptic potentials that these inputs trigger. Indeed I_h activation in response to an *inhibitory postsynaptic potential* implements a “resetting” mechanism for the intrinsic oscillations (Alonso and Dickson, unpublished observations).

As directly demonstrated in this study, a major role of I_h in the stellate cells is that of a “pacemaker” current. This role is not, however, one of providing a “background” depolarizing current that sustains rhythmic discharge but of contributing to the generation of persistent subthreshold oscillatory activity. We confirmed that the bradycardic agent ZD7288 selectively abolished I_h in the range of potentials corresponding to the oscillatory range and found that it progressively and completely abolished the rhythmic subthreshold oscillations. This evidence directly implicates I_h in their generation.

In the present study, we found no evidence of a slow outward K^+ conductance, such as the M current, being necessary for the generation of the subthreshold oscillations by the stellate cells. Indeed Ba^{2+} , which blocks the M-current as well as leak K^+ conductances and I_{Kir} , increased the amplitude of the oscillations, which also became more regular and lower in frequency. This result is in contrast to our previous study using sharp electrodes (Alonso and Klink 1993) in which we reported an apparent block of the oscillations with Ba^{2+} . However, in that study Ba^{2+} was applied in the absence of glutamatergic and GABAergic neurotransmission and thus the apparent block may have been due to the increased synaptic background activity that is caused by Ba^{2+} . In addition, because Ba^{2+} also prolonged the action potential, allowing for an increased Ca^{2+} influx and an enhancement of the slow afterhyperpolarization (see Fig. 10 in Klink and Alonso 1993), it could have been that the apparent block of the oscillations also was related to a membrane shunting effect by a Ca^{2+} -dependent conductance. To test these possibilities, we performed additional sharp electrodes recordings from stellate cells in a similar manner as previously described (Alonso and Klink 1993) in which Ba^{2+} application (1 mM) was conducted in the presence of glutamatergic and GABAergic antagonists and/or the Ca^{2+} channel blocker Co^{2+} (2 mM) ($n = 6$; not shown). In these experiments, in agreement with the above interpretations, the application of Ba^{2+} during synaptic and Ca^{2+} conductance block with Co^{2+} did not abolish the oscillations, which, however, did become slower in frequency (as in our present whole cell patch study). In addition, a similar result was observed when we repeated the preceding experiment using perforated patch recordings (Horn and Marty 1988) ($n = 2$; not shown). We did, however, observe that, using either sharp electrodes or the perforated patch technique, block of Ca^{2+} conductances with Co^{2+} was required to best reproduce our present results using conventional whole cell patch recording. This suggests that a run-down of Ca^{2+} conductances occurred during the conventional whole cell technique that allowed for a better expression of the oscillations in the presence of Ba^{2+} (as seen in Fig. 9).

The modeling analysis that was carried out on the basis of the biophysical properties of I_h and I_{NaP} , as experimentally derived from the stellate cells, indicated that the interplay between these two currents appears sufficient for the generation of persistent subthreshold oscillations in these neurons. First, in the SCs, the G_h activation curve has a rather positive threshold for activation (at about -45 mV) and overlaps sub-

stantially with the G_{NaP} activation curve (threshold at about -65 mV) (Magistretti and Alonso 1999), thus setting the stage for their potential interplay because these conductances activate with voltage changes in opposite directions. Second, and most importantly, the slow activation and deactivation kinetics of I_h implement a delayed feed-back mechanism to the voltage changes led by the “instantaneous” changes in I_{NaP} , thus causing the emergence of sustained oscillatory activity (given the passive membrane properties of the cells). Alonso and Llinás (1989) initially had postulated an interaction between I_h and I_{NaP} as the basis for the generation of subthreshold oscillations by the SCs. Using a bifurcation analysis, White et al. (1995) also had implicated I_h (in addition to an as-yet undetermined slow outward rectifier) in the generation of these oscillations. More recently, an interaction between I_h and I_{NaP} also has been proposed as the basis for subthreshold membrane potential resonance in response to oscillatory intracellular current injection in sensorimotor cortex neurons (Hutcheon et al. 1996a,b). During the last 10 years, Na^+ -dependent subthreshold oscillations similar to those expressed by the SCs also have been observed in other brain neurons (Alonso et al. 1996; Amitai 1994; Gutfreund et al. 1995; Leung and Yim 1991; Llinás et al. 1991; Pape et al. 1998), and they have been considered typically to emerge from the interplay between I_{NaP} and an outward K^+ current, such as an M-type current (Gutfreund et al. 1995; Llinás et al. 1991; Pape et al. 1998; White et al. 1995, 1998) or an A-type current (Wang 1993). As in other situations, distinct ionic mechanisms may be responsible for a similar electrophysiological event in different neurons according to their specific integrative needs.

Interestingly, we found that complete abolition of the rhythmic subthreshold oscillations required a very substantial reduction of I_h . Cs^+ , which we found to reduce I_h by $\sim 65\%$, did not completely abolish the oscillations, thus suggesting that the oscillatory process per se is very robust and rather insensitive to variations in the level of I_h expression. Indeed our simplified model maintained sustained oscillatory activity even when I_h was decreased by 50% (not shown). Further reductions caused a very rapidly damping oscillation. However, in an elaborated SC model that we have constructed (Fransén et al. 1998) when the impact of channel noise (White et al. 1998) is considered, the SC model behaved much more closely to the “real” cell in that I_h reductions of up to $\sim 70\%$ were required to completely abolish subthreshold rhythmicity. The critical importance of stochastic noise in sculpturing the membrane voltage behavior of the SCs has been treated in detail by White et al. (1998).

Functional role of the subthreshold oscillations

The potential functional role of subthreshold oscillatory activity in neocortical neurons was reviewed recently by Connors (Connors and Amitai 1997) and also has been treated in detail by others (Engel et al. 1992; Gray 1994; Laurent 1996; Singer 1993). We will discuss briefly here the potential functional implications that the presence of rhythmic subthreshold oscillatory activity in the stellate cells may have with regard to temporal lobe function. A well-established role of the entorhinal network is that of memory function. Indeed the stellate cells from EC layer II occupy a privileged position in the neocortico-hippocampo-neocortical circuit. They are the targets of convergent information from polysensory associational

areas that they funnel to the dentate gyrus of the hippocampal formation via the perforant path. Thus a potential role of the intrinsic oscillatory activity of the stellate cells could be that of implementing a synchronizing mechanism by which convergent sensory information is coordinated temporally for its transfer to the hippocampal processing machinery and the formation of a memory event (Buzsáki 1989, 1996; Lopes da Silva et al. 1985; Rudell et al. 1980;). Although still far from directly testing such a hypothesis given the complex nature of the information processed by the EC, we do know that EC layer II neurons are powerful generators of theta rhythmicity "in vivo" (Alonso and García-Austt 1987a,b; Dickson et al. 1995; Mitchell and Ranck 1980), and the theta rhythm has been implicated in memory processes (Greenstein et al. 1988; Holscher et al. 1997; Huerta and Lisman 1993; Larson et al. 1986; Lisman and Idiart 1995; Pavlides et al. 1988; Winson 1978). Importantly, analysis of inhibitory synaptic input on EC layer II neurons show that inhibitory postsynaptic potentials, which EC stellates are likely to receive during theta (cf. Fox 1989; Leung and Yim 1986; Soltesz and Deschênes 1993; Ylinen et al. 1995) can very efficiently reset the intrinsic oscillations (Alonso and Dickson, unpublished observations; see also Cobb et al. 1995). In addition, theoretical, experimental and modeling studies also indicate that the intrinsic subthreshold oscillations of individual cells can engender synchronized population activity (Engel et al. 1992; Fransén et al. 1999; Jefferys et al. 1996; König et al. 1995; Lampl and Yarom 1993; Singer 1993; Steriade et al. 1990).

In conclusion, the stellate cells from EC layer II display a very robust I_h current, which, in conjunction with I_{NaP} , gives rise to the expression of rhythmic subthreshold oscillations of the membrane potential in these cells. These oscillations are likely to implement a synchronizing mechanism that may be important in the memory function of the temporal lobe. Understanding the biophysical/molecular and neuromodulatory aspects of the distinct voltage-gated ion channels underlying I_h and I_{NaP} thus may be of critical importance in exploring the functions of the EC network.

We thank I. Manns for kind and efficient aid in Matlab programming.

This study was funded by grants from the Fonds de la Recherche en Santé du Québec and the Medical Research Council (MRC) to A. Alonso and a grant from the Human Frontier Science Program Organization (HFSP) to A. Alonso and M. Hasselmo. C. T. Dickson was supported by fellowships from the National Sciences and Engineering Research Council and the Savoy Foundation. M. H. Shalinsky was supported by a studentship from the MRC. E. Fransén was supported by the Swedish Foundation for International Cooperation in Research in Higher Education (STINT) and the P. E. Lindahls fund.

Address for reprint requests: A. Alonso, Dept. of Neurology and Neurosurgery, McGill University, 3801 University St., Montreal, Quebec H3A 2B4, Canada.

Received 27 August 1999; accepted in final form 11 January 2000.

REFERENCES

- ALONSO, A. AND GARCÍA-AUSTT, E. Neuronal sources of theta rhythm in the entorhinal cortex of the rat. I. Laminar distribution of theta field potentials. *Exp. Brain Res.* 67: 493–501, 1987a.
- ALONSO, A. AND GARCÍA-AUSTT, E. Neuronal sources of theta rhythm in the entorhinal cortex of the rat. II. Phase relations between unit discharges and theta field potentials. *Exp. Brain Res.* 67: 502–509, 1987b.
- ALONSO, A., KHATEB, A., FORT, P., JONES, B. E., AND MUHLETHALER, M. Differential oscillatory properties of cholinergic and non-cholinergic neurons in the nucleus basalis. *Eur. J. Neurosci.* 8: 169–182, 1996.
- ALONSO, A. AND KLINK, R. Differential electroresponsiveness of stellate and pyramidal-like cells of medial entorhinal cortex layer II. *J. Neurophysiol.* 70: 128–143, 1993.
- ALONSO, A. AND LLINÁS, R. R. Subthreshold Na^+ -dependent theta-like rhythmicity in stellate cells of entorhinal cortex layer II. *Nature* 342: 175–177, 1989.
- AMITAI, Y. Membrane potential oscillations underlying firing patterns in neocortical neurons. *Neuroscience* 63: 151–161, 1994.
- BAKER, K., WARREN, K. S., YELLEN, G., AND FISHMAN, M. C. Defective "pacemaker" current (I_h) in a zebrafish mutant with a slow heart rate. *Proc. Natl. Acad. Sci. USA* 94: 4554–4559, 1997.
- BAL, T. AND McCORMICK, D. A. Synchronized oscillations in the inferior olive are controlled by the hyperpolarization-activated cation current. *J. Neurophysiol.* 77: 3145–3156, 1997.
- BAYLISS, D. A., VIANA, F., BELLINGHAM, M. C., AND BERGER, A. J. Characteristics and postnatal development of a hyperpolarization-activated inward current in rat hypoglossal motoneurons in vitro. *J. Neurophysiol.* 71: 119–128, 1994.
- BO SMITH, R. E., BRIGGS, I., AND STURGEON, N. C. Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (I_h) in guinea-pig dissociated sinoatrial node cells. *Br. J. Pharmacol.* 110: 343–349, 1993.
- BROWN, H. AND DiFRANCESCO, D. Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *J. Physiol. (Lond.)* 308: 331–351, 1980.
- BUZSÁKI, G. Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience* 31: 551–570, 1989.
- BUZSÁKI, G. The hippocampo-neocortical dialogue. *Cereb. Cortex* 6: 81–92, 1996.
- CHAMPIGNY, G. AND LENFANT, J. Block and activation of the hyperpolarization-activated inward current by Ba and Cs in frog sinus venosus. *Pflügers Arch. Eur. J. Physiol.* 407: 684–690, 1986.
- CLAPHAM, D. E. Not so funny anymore: pacing channels are cloned. *Neuron* 21: 5–7, 1998.
- COBB, S. R., BUHL, E. H., HALASY, K., PAULSEN, O., AND SOMOGYI, P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378: 75–78, 1995.
- CONNORS, B. W. AND AMITAI, Y. Making waves in the neocortex. *Neuron* 18: 347–349, 1997.
- CREPEL, F. AND PENIT-SORIA, J. Inward rectification and low threshold calcium conductance in rat cerebellar purkinje cells. An in vitro study. *J. Physiol. (Lond.)* 372: 1–23, 1986.
- CRILL, W. E. Persistent sodium current in mammalian central neurons. *Annu. Rev. Physiol.* 58: 349–362, 1996.
- DICKSON, C. T. AND ALONSO, A. Hyperpolarization-activated current (I_h) in layer II cells of the medial entorhinal cortex. *Soc. Neurosci. Abstr.* 22: 1027, 1996.
- DICKSON, C. T. AND ALONSO, A. Role of I_h in the generation of subthreshold membrane potential oscillations (MPOs) of entorhinal cortex (EC) layer II stellate cells. *Soc. Neurosci. Abstr.* 24: 2035, 1998.
- DICKSON, C. T., KIRK, I. J., ODDIE, S. D., AND BLAND, B. H. Classification of theta-related cells in the entorhinal cortex: cell discharges are controlled by the ascending brainstem synchronizing pathway in parallel with hippocampal theta-related cells. *Hippocampus* 5: 306–319, 1995.
- DICKSON, C. T., MENA, A. R., AND ALONSO, A. Electroresponsiveness of medial entorhinal cortex layer III neurons in vitro. *Neuroscience* 81: 937–950, 1997.
- DiFRANCESCO, D. A new interpretation of the pace-maker current in calf Purkinje fibers. *J. Physiol. (Lond.)* 314: 359–376, 1981.
- DiFRANCESCO, D. Pacemaker mechanisms in cardiac tissue. *Annu. Rev. Physiol.* 55: 455–472, 1993.
- DiFRANCESCO, D., FERRONI, M., MAZZANTI, M., AND TROMBA, C. Properties of the hyperpolarizing-activated current (I_h) in cells isolated from the rabbit sino-atrial node. *J. Physiol. (Lond.)* 377: 61–88, 1986.
- DOLORFO, C. L. AND AMARAL, D. G. Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. *J. Comp. Neurol.* 398: 25–48, 1998.
- ENGEL, A. K., KÖNIG, P., KREITER, A. K., SCHILLEN, T. B., AND SINGER, W. Temporal coding in the visual cortex: new vistas on integration in the nervous system. *Trends Neurosci.* 15: 218–226, 1992.
- FINCH, D. M., TAN, A. M., AND ISOKAWA-AKESON, M. Feedforward inhibition of the rat entorhinal cortex and subicular complex. *J. Neurosci.* 8: 2213–2226, 1988.

- FOX, S. E. Membrane potential and impedance changes in hippocampal pyramidal cells during theta rhythm. *Exp. Brain Res.* 77: 283–294, 1989.
- FRANSÉN, E., DICKSON, C. T., MAGISTRETTI, J., ALONSO, A., AND HASSELMO, M. E. Modeling the generation of subthreshold membrane potential oscillations of entorhinal cortex layer II stellate cells. *Soc. Neurosci. Abstr.* 24: 814.815, 1998.
- FRANSÉN, E., WALLENSTEIN, G. V., ALONSO, A., DICKSON, C. T., AND HASSELMO, M. E. A biophysical simulation of intrinsic and network properties of entorhinal cortex. *Neurocomputing* 26–27: 375–380, 1999.
- GASPARINI, S. AND DI FRANCESCO, D. Action of the hyperpolarization-activated current (I_h) blocker ZD 7288 in hippocampal CA1 neurons. *Pflügers Arch. Eur. J. Physiol.* 435: 99–106, 1997.
- GAUSS, R., SEIFERT, R., AND KAUPP, U. B. Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. *Nature* 393: 583–587, 1998.
- GRAY, C. M. Synchronous oscillations in neuronal systems: mechanisms and functions. *J. Comput. Neurosci.* 1: 11–38, 1994.
- GREENSTEIN, Y. J., PAVLIDES, C., AND WINSON, J. Long-term potentiation in the dentate gyrus is preferentially induced at theta rhythm periodicity. *Brain Res.* 438: 331–334, 1988.
- GUTFREUND, Y., YAROM, Y., AND SEGEV, I. Subthreshold oscillations and resonant frequency in guinea-pig cortical neurons: physiology and modeling. *J. Physiol. (Lond.)* 483: 621–640, 1995.
- HAGIWARA, S., MIYAZAKI, S., MOODY, W., AND PATLAK, J. Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. *J. Physiol. (Lond.)* 279: 167–185, 1978.
- HAGIWARA, S., MIYAZAKI, S., AND ROSENTHAL, N. P. Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. Gen. Physiol.* 67: 621–638, 1976.
- HALLIWELL, J. V. AND ADAMS, P. R. Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Res.* 250: 71–92, 1982.
- HARRIS, N. C. AND CONSTANTIN, A. Mechanism of block by ZD 7288 of the hyperpolarization-activated inward rectifying current in guinea pig substantia nigra neurons in vitro. *J. Neurophysiol.* 74: 2366–2378, 1995.
- HILLE, B. *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer Assoc., 1992.
- HOLSCHER, C., ANWYL, R., AND ROWAN, M. J. Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can be depotentiated by stimulation on the negative phase in area CA1 in vivo. *J. Neurosci.* 17: 6470–6477, 1997.
- HOPFIELD, J. J. Pattern recognition computation using action potential timing for stimulus representation. *Nature* 376: 33–36, 1995.
- HORN, R. AND MARTY, A. Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J. Gen. Physiol.* 92: 145–159, 1988.
- HUERTA, P. T. AND LISMAN, J. E. Heightened synaptic plasticity of hippocampal CA1 neurones during a cholinergically induced rhythmic state. *Nature* 364: 723–725, 1993.
- HUTCHEON, B., MIURA, R. M., AND PUIL, E. Models of subthreshold membrane resonance in neocortical neurons. *J. Neurophysiol.* 76: 698–714, 1996a.
- HUTCHEON, B., MIURA, R. M., AND PUIL, E. Subthreshold membrane resonance in neocortical neurons. *J. Neurophysiol.* 76: 683–697, 1996b.
- ISHII, T. M., TAKANO, M., XIE, L.-H., NOMA, A., AND OHMORI, H. Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. *J. Biol. Chem.* 274: 12835–12839, 1999.
- JEFFERYS, J., TRAUB, R., AND WHITTINGTON, M. Neuronal networks for induced “40 Hz” rhythms. *Trends Neurosci.* 19: 202–208, 1996.
- JONES, R.S.G. Synaptic and intrinsic properties of neurons of origin of the perforant path in layer II of the rat entorhinal cortex in vitro. *Hippocampus* 4: 335–353, 1994.
- JONES, R.S.G. AND BUHL, E. H. Basket-like interneurons in layer II of the entorhinal cortex exhibit a powerful NMDA-mediated synaptic excitation. *Neurosci. Lett.* 149: 35–39, 1993.
- KAMONDI, A. AND REINER, P. B. Hyperpolarization-activated inward current in histaminergic tuberomammillary neurons of the rat hypothalamus. *J. Neurophysiol.* 66: 1902–1911, 1991.
- KLINK, R. AND ALONSO, A. Ionic mechanisms for the subthreshold oscillations and differential electroresponsiveness of medial entorhinal cortex layer II neurons. *J. Neurophysiol.* 70: 144–157, 1993.
- KLINK, R. AND ALONSO, A. Morphological characteristics of layer II projection neurons in the rat medial entorhinal cortex. *Hippocampus* 7: 571–583, 1997.
- KOHLER, C. Cytochemical architecture of the entorhinal area. *Adv. Exp. Med. Biol.* 203: 83–98, 1988.
- KOHLER, C., CHAN-PALAY, V., AND WU, J.-Y. Neurons and terminals in the retrohippocampal region in the rat’s brain identified by anti- γ -aminobutyric acid and anti-glutamic acid decarboxylase immunocytochemistry. *Anat. Embryol.* 173: 35–44, 1985.
- KÖNIG, P., ENGEL, A. K., AND SINGER, W. Relation between oscillatory activity and long-range synchronization in cat visual cortex. *Proc. Natl. Acad. Sci. USA* 92: 290–294, 1995.
- LAMPL, I. AND YAROM, Y. Subthreshold oscillations of the membrane potential: a functional synchronizing and timing device. *J. Neurophysiol.* 70: 2181–2186, 1993.
- LARSON, J., WONG, D., AND LYNCH, G. Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. *Brain Res.* 368: 347–350, 1986.
- LAURENT, G. Dynamical representation of odors by oscillating and evolving neural assemblies. *Trends Neurosci.* 19: 489–496, 1996.
- LEUNG, L.-W.S. AND YIM, C. Y. Intracellular records of theta rhythm in hippocampal CA1 cells of the rat. *Brain Res.* 367: 323–327, 1986.
- LEUNG, L.-W.S. AND YIM, C.-Y.C. Intrinsic membrane potential oscillations in hippocampal neurons in vitro. *Brain Res.* 553: 261–274, 1991.
- LISMAN, J. E. AND IDIART, M.A.P. Storage of 7 ± 2 short term memories in oscillatory subcycles. *Science* 267: 1512–1515, 1995.
- LLINÁS, R. R. The intrinsic electrophysiological properties of mammalian neurons: Insights into central nervous system function. *Science* 242: 1654–1664, 1988.
- LLINÁS, R. R., GRACE, A. A., AND YAROM, Y. In vitro neurons in mammalian cortical layer 4 exhibit intrinsic oscillatory activity in the 10- to 50-Hz frequency range. *Proc. Natl. Acad. Sci. USA* 88: 897–901, 1991.
- LLINÁS, R. AND JAHNSEN, H. Electrophysiology of mammalian thalamic neurones in vitro. *Nature* 297: 406–408, 1982.
- LOPES DA SILVA, F. H., GROENEWEGER, H. J., HOSHEIMER, J., ROOM, P., WITTER, M. P., VAN GROEN, T., AND WADMAN, W. J. The hippocampus as a set of partially overlapping segments with a topographically organized system of inputs and outputs: the entorhinal cortex as a sensory gate, the medial septum as a gain-setting system and the ventral striatum as a motor interface. In: *Electrical Activity of the Archicortex*, edited by G. Buzsáki and C. H. Vanderwolf. Budapest: Akademiai Kiado, 1985, p. 83–106.
- LUDWIG, A., ZONG, X., JEGELITSCH, M., HOFMANN, F., AND BIEL, M. A family of hyperpolarization-activated mammalian cation channels. *Nature* 393: 587–591, 1998.
- LUDWIG, A., ZONG, X., STIEBER, J., HULLIN, R., HOFMANN, F., AND BIEL, M. Two pacemaker channels from human heart with profoundly different activation kinetics. *EMBO J.* 18: 2323–2329, 1999.
- LÜTHI, A., BAL, T., AND MCCORMICK, D. A. Periodicity of thalamic spindle waves is abolished by ZD7288, a blocker of I_h. *J. Neurophysiol.* 79: 3284–3289, 1998.
- LÜTHI, A. AND MCCORMICK, D. A. H-current: properties of a neuronal and network pacemaker. *Neuron* 21: 9–12, 1998.
- MACCAFFERRI, G., MANGONI, M., LAZZARI, A., AND DI FRANCESCO, D. Properties of the hyperpolarization-activated current in rat hippocampal CA1 pyramidal cells. *J. Neurophysiol.* 69: 2129–2136, 1993.
- MACCAFFERRI, G. AND MCBAIN, C. J. The hyperpolarization-activated current (I_h) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurons. *J. Physiol. (Lond.)* 497: 119–130, 1996.
- MAGISTRETTI, J. AND ALONSO, A. Biophysical properties and slow voltage-dependent inactivation of a sustained sodium current in entorhinal cortex layer II principal neurons. A whole-cell and single-channel study. *J. Gen. Physiol.* 114: 491–509, 1999.
- MAGISTRETTI, J., RAGSDALE, D. S., AND ALONSO, A. High conductance sustained single channel activity responsible for the low threshold persistent Na⁺ current in entorhinal cortex neurons. *J. Neurosci.* 19: 7334–7341, 1999.
- MAYER, M. L. AND WESTBROOK, G. L. A voltage-clamp analysis of inward (anomalous) rectification in mouse spinal sensory ganglion neurones. *J. Physiol. (Lond.)* 340: 19–45, 1983.
- MCCORMICK, D. A. AND PAPE, H.-C. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *J. Physiol. (Lond.)* 431: 291–318, 1990.
- MERCURI, N. B., BONCI, A., CALABRESI, P., STEFANI, A., AND BERNARDI, G. Properties of the hyperpolarization-activated cation current I_h in rat mid-brain dopaminergic neurones. *Eur. J. Neurosci.* 7: 462–469, 1995.
- MITCHELL, S. J. AND RANCK, J.B.J. Generation of theta rhythm in medial entorhinal cortex of freely moving rats. *Brain Res.* 189: 49–66, 1980.
- NEHER, E. Correction of liquid junction potentials in patch clamp experiments. *Methods Enzymol.* 207: 123–131, 1992.
- NOMA, A. AND IRISAWA, H. A time- and voltage-dependent potassium current in the rabbit sinoatrial node cell. *Pflügers Arch.* 366: 251–258, 1976.

- PAPE, H.-C. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu. Rev. Physiol.* 58: 299–327, 1996.
- PAPE, H.-C., PARE, D., AND DRIESANG, R. B. Two types of intrinsic oscillations in neurons of the lateral and basolateral nuclei of the amygdala. *J. Neurophysiol.* 79: 205–216, 1998.
- PAVLIDES, C., GREENSTEIN, Y., GRUDMAN, M., AND WINSON, J. Long-term potentiation in the dentate gyrus is induced preferentially on the positive phase of theta-rhythm. *Brain Res.* 439: 383–387, 1988.
- RAMON Y CAJAL, S. Sobre un ganglio especial de la corteza eseno-occipital. *Trab. del Lab. de Invest. Biol. Univ. Madrid* 1: 189–201, 1902.
- RUDELL, A. P., FOX, S. E., AND RANCK, J. B. Hippocampal excitability phase-locked to the theta rhythm in walking rats. *Exp. Neurol.* 68: 87–96, 1980.
- SANTORO, B., LIU, D. T., YAO, H., BARTSCH, D., KANDEL, E. R., SIEGELBAUM, S. A., AND TIBBS, G. R. Identification of a gene encoding a hyperpolarization-activated pacemaker channel in the brain. *Cell* 93: 717–729, 1998.
- SINGER, W. Synchronization of cortical activity and its putative role in information processing and learning. *Annu. Rev. Physiol.* 55: 349–374, 1993.
- SOLOMON, J. S. AND NERBONNE, J. M. Two kinetically distinct components of hyperpolarization-activated current in rat superior colliculus-projecting neurons. *J. Physiol. (Lond.)* 469: 291–313, 1993.
- SOLTESZ, I. AND DESCHÊNES, M. Low- and high-frequency membrane potential oscillations during theta activity in CA1 and CA3 pyramidal neurons of the rat hippocampus under ketamine-xylazine anesthesia. *J. Neurophysiol.* 70: 97–116, 1993.
- SOLTESZ, I., LIGHTOWLER, S., LERESCHE, N., JASSIK-GERSCHENFELD, D., POLLARD, C. E., AND CRUNELLI, V. Two inward currents and the transformation of low-frequency oscillations of rat and cat thalamocortical cells. *J. Physiol. (Lond.)* 441: 175–197, 1991.
- SPAIN, W. J., SCHWINDT, P. C., AND CRILL, W. E. Anomalous rectification in neurons from cat sensorimotor cortex in vitro. *J. Neurophysiol.* 57: 1555–1576, 1987.
- STERIADE, M., GLOOR, P., LLINÁS, R. R., LOPES DA SILVA, F. H., AND MESULAM, M.-M. Basic mechanisms of cerebral rhythmic activities. *EEG Clin. Neurophysiol.* 76: 481–508, 1990.
- STERIADE, M. AND LLINÁS, R. R. The functional states of the thalamus and the associated neuronal interplay. *Physiol. Rev.* 68: 649–742, 1988.
- STERIADE, M., MCCORMICK, D. A., AND SEJNOWSKI, T. J. Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262: 679–685, 1993.
- TAKAHASHI, T. Inward rectification in neonatal rat spinal motoneurons. *J. Physiol. (Lond.)* 423: 57–62, 1990.
- TRAVAGLI, R. A. AND GILLIS, R. A. Hyperpolarization-activated currents, I_h , and I_{kir} , in rat dorsal motor nucleus of the vagus neurons in vitro. *J. Neurophysiol.* 71: 1308–1317, 1994.
- WANG, X.-J. Ionic basis for intrinsic 40 Hz neuronal oscillations. *Neuroreport* 5: 221–224, 1993.
- WHITE, J. A., BUDDE, T., AND KAY, A. R. A bifurcation analysis of neuronal subthreshold oscillations. *Biophys. J.* 69: 1203–1217, 1995.
- WHITE, J. A., KLINK, R., ALONSO, A., AND KAY, A. R. Noise from voltage-gated ion channels may influence neuronal dynamics in the entorhinal cortex. *J. Neurophysiol.* 80: 262–269, 1998.
- WILLIAMS, S. R., TURNER, J. P., HUGHES, S. W., AND CRUNELLI, V. On the nature of anomalous rectification in thalamocortical neurones of the cat ventrobasal thalamus in vitro. *J. Physiol. (Lond.)* 505: 727–747, 1997.
- WINSON, J. Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science* 201: 160–163, 1978.
- WOUTERLOOT, P. G., HÄRTIG, W., BRÜCKNER, G., AND WITTER, M. P. Parvalbumin-immunoreactive neurons in the entorhinal cortex of the rat: localization, morphology, connectivity and ultrastructure. *J. Neurocytol.* 24: 135–153, 1995.
- YANAGIHARA, K. AND IRISAWA, H. Inward current activated during hyperpolarization in the rabbit sino atrial node cell. *Pfügers Arch. Eur. J. Physiol.* 385: 11–19, 1980.
- YLINEN, A., SOLTESZ, I., BRAGIN, A., PENTTONEN, M., SIK, A., AND BUZSÁKI, G. Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus* 5: 78–90, 1995.