SUMMARY AND CONCLUSIONS

1. We obtained whole cell data from sensorimotor cortical neurons of in vitro slices (juvenile rats) and observed a low frequency resonance (1–2 Hz) in their voltage responses. We constructed models of subthreshold membrane currents to determine whether a hyperpolarization-activated cation current ($I_{\text{h}}$) is sufficient to account for this resonance.

2. Parameter values for a basic $I_{\text{h}}$ (BH) model were estimated from voltage-clamp experiments at room temperature. The BH model formed a component of a reduced membrane (RM) model. On numerical integration, the RM model exhibited voltage sags and rebounds to injected current pulses; maximal voltage responses to injected oscillatory currents occurred near 2 Hz.

3. We compared the experimentally measured frequency-response curves (FRCs) at room temperature with the theoretical FRCs derived from the RM model. The theoretical FRCs exhibited resonant humps with peaks between 1 and 2 Hz. At 36°C, the theoretical FRCs peaked near 10 Hz. The characteristics of theoretical and observed FRCs were in close agreement, demonstrating that $I_{\text{h}}$ is sufficient to cause resonance. Thus we classified $I_{\text{h}}$ as a resonator current.

4. We developed a technique, the reactive current clamp (RCC), to inject a computer-calculated current corresponding to a membrane ionic current in response to the membrane potential of the neuron. This enabled us to study the interaction of an artificial ionic current with living neurons (electronic pharmacology or EP method). Using the RCC, a simplified version of the BH model was used to cancel an endogenous $I_{\text{h}}$ (electronic antagonism) and to introduce an artificial $I_{\text{h}}$ (electronic expression) when an endogenous $I_{\text{h}}$ was absent. Antagonism of $I_{\text{h}}$ eliminated sags and rebounds, whereas expression of $I_{\text{h}}$ endowed neurons with resonance and the frequency-selective firing that accompanies resonance in neurons with an endogenous $I_{\text{h}}$. Previous investigations have relied on the specificity of pharmacological agents to block ionic channels, e.g., $\text{Cs}^+$ to block $I_{\text{h}}$. However, $\text{Cs}^+$ additionally affects other currents. This represents the first time an in vitro modeling technique (RCC) has been used to antagonize a specific endogenous current, $I_{\text{h}}$.

5. A simplified RM model yielded values of the resonant frequency and $Q$ (an index of the sharpness of resonance), which rose almost linearly between −55 and −80 mV. Resonant frequencies could be much higher than $f_{\text{res}} = (2\pi \tau_{\text{h}}) − 1$ where $\tau_{\text{h}}$ is the activation time constant for $I_{\text{h}}$.

6. In the FRCs, resonance appeared as a hump at intermediate frequencies because of low- and high-frequency attenuations due to $I_{\text{h}}$ and membrane capacitance, respectively. Changing the parameters of $I_{\text{h}}$ altered the low-frequency attenuation and, hence, the resonance. Changes in the leak conductance affected both the low- and high-frequency attenuations.

7. We modeled an inwardly rectifying $K^+$ current ($I_{\text{IR}}$) and a persistent $\text{Na}^+$ current ($I_{\text{nap}}$) to study their effects on resonance. Neither current produced resonance in the absence of $I_{\text{h}}$. We found that $I_{\text{h}}$ attenuated, whereas $I_{\text{nap}}$ amplified resonance. Thus $I_{\text{h}}$ and $I_{\text{nap}}$ are classified as attenuator and amplifier currents, respectively.

8. Resonators and attenuators differ in that they have longer and shorter time constants, respectively, compared with the membrane time constant. Therefore an increase in the leak conductance decreases the membrane time constant, which can transform an attenuator into a resonator, altering the frequency response. This suggests a novel mechanism for modulating the frequency responses of neurons to inputs.

9. These investigations have provided a theoretical framework for detailed understanding of mechanisms that produce resonance in cortical neurons. Resonance is one aspect of the intrinsic rhythmicity of neurons. The rhythmicity due to $I_{\text{h}}$ is latent until it is revealed by oscillatory inputs. The rhythmicity may become evident as spontaneous oscillations near the resonant frequency if the resonance is amplified by a current such as $I_{\text{nap}}$. We suggest that these intrinsic properties could play a role in the propagation of coordinated rhythmic activity in central neural circuits. A specific consequence of $I_{\text{h}}$ resonance could be a stabilization of the frequency of cortical activity near 10 Hz.

INTRODUCTION

Subthreshold neuronal currents contribute to the rhythmicity and synchronization of electrical activity in the mammalian brain. For instance, a low-threshold calcium current ($I_{\text{f}}$) in thalamocortical neurons contributes to the synchronization of activity during sleep. Here, rhythmic inhibitory inputs from the reticular nucleus result in burst of spikes by a postinhibitory rebound mechanism (Steriade et al. 1993). In neocortical neurons, another subthreshold current, the hyperpolarization-activated $\text{Na}^+$/K$^+$ current ($I_{\text{h}}$), has been implicated in the production of postinhibitory rebounds following periods of hyperpolarization (Solomon and Nerbonne 1993a,b; Spain et al. 1991). However, it is unknown how $I_{\text{h}}$ promotes rhythmic activity in neocortical neurons.

In a previous study, we determined that $I_{\text{h}}$ produces a subthreshold frequency preference in neurons of rat sensorimotor cortex (Hutcheon et al. 1996). This frequency preference appeared as a membrane resonance with a peak between 0.5 and 5 Hz in approximately two-thirds of regular spiking (RS) and intrinsic bursting (IB) neurons in response to subthreshold inputs. In response to suprathreshold inputs, the frequency preference was translated into frequency-selective firing, i.e., an increased likelihood of firing when the stimulation was near the resonant frequency. We also demonstrated that $I_{\text{h}}$ is necessary for the generation of resonance because blockade of $I_{\text{h}}$ with external $\text{Cs}^+$ also blocked resonance. However, this did not eliminate the possibility that other conductances are necessary for resonance or may alter its frequency or magnitude.

In the present study, we seek to clarify the relationship between resonance and the kinetic and steady state properties
of $I_\text{H}$ through a combination of experiment and mathematical modeling. We use AC analysis (DeFelice 1981; Puil et al. 1986, 1987) to characterize the responses of real and model neurons to inputs at specific frequencies. For individual neurons, we compare their experimentally measured and theoretically predicted frequency-response curves (FRCs) to assess the contribution of $I_\text{H}$ to resonance. Because the model that generates the theoretical FRC contains only $I_\text{H}$, a leak current ($I_{\text{leak}}$), and a capacitive current ($I_{\text{cap}}$), good agreement between the observed and theoretical FRCs would indicate that $I_\text{H}$ is the major contributor to resonance.

We also use a new technique, the reactive current clamp (RCC) to show that the presence of a simulated $I_\text{H}$ in a real neuron causes resonance and frequency-selective firing. The RCC, which creates a hybrid mathematical-biological model was developed by Sharp et al. (1993a,b; dynamic clamp) and, independently, by us (Hutcheon and Puil 1993; Puil and Hutcheon 1994). Here, we use it primarily to couple Hodgkin-Huxley-type (HH-type) models of voltage-dependent currents to neurons and, for the first time, to electronically cancel the effects of an endogenous current in a neuron.

Finally, we examine how resonance in these models is altered by changes in $I_{\text{leak}}$ and two voltage-dependent currents: the persistent Na+ current ($I_{\text{NaP}}$) (Alzheimer et al. 1993, Staffstrom et al. 1985) and an inwardly rectifying K+ current ($I_{\text{IR}}$, Constanti and Galvan 1983). Both $I_{\text{NaP}}$ and $I_{\text{IR}}$ are present in most neocortical neurons and were shown in the preceding paper to have activation ranges that sometimes overlap with that of $I_\text{H}$ (Hutchison et al. 1996). In that study, their coactivation with $I_\text{H}$ amplified or attenuated resonance but did not result in large changes in the resonant frequency. The interactions of these currents with $I_\text{H}$ and the passive membrane properties create a subthreshold filtering of incoming signals that can influence the computational abilities of neurons.

**METHODS**

The methods for surgical dissection, preparations for in vitro recording, and drug application have been described previously (Hutchison et al. 1996). The use of swept-sine-wave (ZAP) current inputs and frequency domain analysis to characterize the frequency-responses of neurons also has been described (Puil et al. 1986). In this paper, we focus on the magnitudes of neuronal responses to periodic stimulation. The phase information generated by the frequency-domain analysis is therefore not presented.

**Reactive current clamp: theory**

The RCC is a general technique for interactively coupling an arbitrary computer algorithm to a cell (Hutchison and Puil 1993; Puil and Hucheson 1994; Sharp et al. 1993a,b). We will refer to the study of the electrical behavior of cells using the RCC technique, e.g., to mimic an ionic current, as *electronic pharmacology* (EP-method). In this section, we describe an application of this technique whereby the computer contains a model of a voltage-dependent ionic current and the cell is a neuron. In this case, the input to the computer is the membrane voltage of the neuron and the input to the neuron is an injected current that is generated by the computer in response to the voltage.

First, consider the process that results in changes in the membrane voltage of a neuron on injection of a current. Assuming that the neuron is isopotential, the relationship between the capacitive, ionic, and injected currents is

$$\frac{dV}{dt} = -[I_1 + I_2 + \cdots + I_n] + I_{\text{inj}} \quad (1)$$

Here, $V$ is voltage, $c_n$ is the total membrane capacitance, $I_1, \ldots, I_n$ are currents flowing through ion channels, and $I_{\text{inj}}$ is an injected current under the control of the investigator. The use of the RCC technique for electronic pharmacology requires injecting a current that has the time- and voltage-dependent properties of a membrane ionic current into a neuron. This corresponds to adding an extra term, $-I_{\text{RCC}}$, to Eq. 1. Although this current is part of $I_{\text{inj}}$, we can rewrite Eq. 1 so that it is expressed in the same way as one of the neuron's endogenous currents

$$\frac{dV}{dt} = -[I_1 + I_2 + \cdots + I_n + I_{\text{RCC}}] + I_{\text{inj}}(t) \quad (2)$$

where $I_{\text{inj}}(t)$ is an explicit function of specified time $t$ (e.g., a current pulse). The total injected current $I_{\text{inj}}$ of Eq. 1, therefore, is given by

$$I_{\text{inj}} = -I_{\text{RCC}} + I_{\text{inj}}(t). \quad (3)$$

To control the time- and voltage-dependence of $I_{\text{RCC}}$, a number of different models of ionic currents are available. In this paper, we have used a simple model of a nonactivating current with HH-type dynamics with the form

$$I_{\text{RCC}} = g_m[V - V_{\text{rev}}] \quad (4)$$

where $g$ is the maximal conductance, $V_{\text{rev}}$ is an appropriate reversal potential, $m$ is the activation variable, and the value of the exponent, $r$, depends on the current to be simulated. The kinetics for $m$ are given by

$$\frac{dm}{dt} = \frac{m(V) - m(V)}{\tau(V)} \quad (5)$$

where $m(V)$ and $\tau(V)$ describe the voltage dependencies of the steady state activation and the activation time constant, respectively. The functional forms for $m(V)$ and $\tau(V)$ are given in RESULTS.

The RCC technique instructs the computer to integrate Eq. 5 numerically. Then the result of this calculation is used in Eq. 4 to compute $I_{\text{RCC}}$ for injection into the neuron. Because the computations are performed in real time, the interaction between $I_{\text{RCC}}$ and the membrane ionic currents of the neuron approximates the relationship between a naturally occurring voltage-dependent current and the other ionic currents. As a result, the neuron behaves as though it possesses a new ionic current. By analogy with the expression of a cloned ionic current in a cell, we call this process the *electronic expression* of the modeled current within the neuron.

Examination of Eq. 2 shows that the electronically expressed current, $I_{\text{RCC}}$, can be chosen to augment or curtail one of the neuron's endogenous currents. This requires matching the time- and voltage-dependent properties of $I_{\text{RCC}}$ (Eqs. 4 and 5) to the properties of a selected endogenous current so that their terms in Eq. 2 either add or cancel. We call these procedures *electronic agonism* and *electronic antagonism*, respectively, by analogy with pharmacological agonism and antagonism. In the present paper, we first electronically antagonize $I_\text{H}$ in neocortical neurons to validate the $I_\text{H}$ model derived below. We then electronically express the $I_\text{H}$ model in neurons that have no endogenous $I_\text{H}$ to study their firing behavior.
In this section, we will describe the implementation of the RCC as shown schematically in Fig. 1. We used a patch-clamp electrode in the whole cell configuration, connected to the bridge circuit of an Axoclamp 2A amplifier, to sense the membrane voltage and inject current. Specially written software running on a 33 MHz 386 computer directed the periodic sampling of the membrane voltage of the neuron and digitized the voltage values using a 40-kHz analog-to-digital converter (Scientific Solutions). The computer used the sampled values of the voltage to calculate $m$ as described below. Then, $m'$, was fed through a digital-to-analog converter into a four-quadrant multiplier where it was multiplied by a driving force. The driving force, calculated by a differential amplifier, is equal to the difference between the membrane voltage and a preset $V_m$. A voltage divider was used to scale the output from the multiplier and, therefore, played a role corresponding to the maximal conductance of the RCC current (see $g$ in Fig. 1). The analog part of the system (differential amplifier, multiplier, and voltage divider) had a bandwidth >50 kHz; its purpose was to offload calculations involving rapid changes in driving force from the computer.

During the implementation of the RCC, the continuously changing membrane voltage of the neuron was sampled at time intervals of width $\Delta t$ and delivered to the computer as the input for the numerical integration of Eq. 5. The output of the computer’s calculation, $m$, was also delivered as a sequence of discrete values at intervals of $\Delta t$. As shown in Fig. 2, during the interval from time $t_n$ to time $t_{n+1}$, the input to the computer is $V_n$ and its output is $m_n$. The problem in finding a suitable procedure for computing $m_n$ is to predict the change in $m$ from $t_n$ to $t_{n+1}$. For this purpose, an exact solution for the response to a voltage step is available in the special case of Eq. 5. Referring to Fig. 2, the value of $m_n$ is the value of $m$ predicted for time $t_{n+1}$, given $m_{n-1}$ and $V_n$. Thus the formula for updating $m$ was

$$m_n = m_{n-1} + [m'(V_n) - m_{n-1}](1 - e^{-\Delta t/V_m})$$  \hspace{1cm}  \hspace{1cm} (6)

This value then was used to determine $I_{RCC}$ for the interval between $t_n$ and $t_{n+1}$ (see Fig. 2) according to

$$I_{RCC}(t) = g m_n[V(t) - V_m]$$  \hspace{1cm}  \hspace{1cm} (7)

The functions $m'(V)$ and $e^{-\Delta t/V_m}$ were evaluated at various voltages.
dures worked well for all values of ages over a suitable voltage range and stored in arrays for use as lookup tables during on-line calculations. In practice, these procedures worked well for all values of $\Delta t < \tau$.

The minimum time needed to go once around the RCC feedback loop was 200 $\mu$s. We often specified longer cycle times but always insured that $\Delta t/\tau$ was small. It was not necessary to make $\Delta t$ small relative to the fast changes in the membrane voltage because the calculations for the driving force were handled by the analog part of the system.

The RCC depends crucially on the accurate measurement of membrane potential. However, the use of bridge mode for single-electrode voltage recording and current injection sometimes resulted in small measurement errors. These were minimized by using in-slice, whole cell patch recording; nevertheless, a maximum uncompensated series resistance of 10 $M\Omega$ sometimes was present during RCC current injections of $\pm 300 \ pA$. This resulted in a maximum error of 3 mV, which was small compared with the reciprocal of the maximum slope of the activation curve of the theoretical conductance. Electrode drift of comparable magnitude sometimes developed. When the uncompensated drift was $>3 \ mV$, the cells were not used for analysis.

**ELECTRONIC ANTAGONISM—SUBTRACTION OF AN ENDOGENOUS CURRENT.** As a demonstration of electronic antagonism, we used the RCC to electronically cancel an endogenous $I_z$ (see RESULTS). We initially used parameter values for the model $I_z$ that were approximately correct according to previous experience. Then it was necessary to bring these values into accordance with the actual properties of the endogenous current. This was accomplished by repeatedly subjecting the hybrid neuron-computer system to hyperpolarizing and depolarizing pulses and adjusting the parameters of the model until the sags and rebounds characteristic of $I_z$ were abolished over the activation range of $I_z$.

**ELECTRONIC EXPRESSION—INSERTION OF AN ARTIFICIAL CURRENT.** As a demonstration of electronic expression, we used the RCC to electronically produce $I_z$. Figure 3A shows the effects of inserting an artificial $I_z$ current into a model cell ($500 \ M\Omega$ resistor in parallel with a 33-pF capacitor). The artificial $I_z$ produced sags and rebounds in the voltage responses to rectangular current pulse inputs. This was reflected in a nonohmic current-voltage ($I-V$) relationship. Because the model cell is electrically passive, all nonohmic behaviors are due to the electronic $I_z$ inserted by the RCC. Figure 3B shows that the electronic expression of a model $I_z$ in an actual neocortical neuron changes the subthreshold $I-V$ relationship in a similar way.

**RESULTS**

In this paper, we construct several mathematical models of the subthreshold electrical responsiveness of neocortical neurons with the aim of explaining the resonance often seen in their FRCS. In these models, we make the simplifying assumption that the main factors determining the resonant properties of neurons near their resting potentials are their passive characteristics (capacitance and leak conductance) and the properties of $I_z$.

The models used in this paper include: a model of the process of $I_z$ activation [the basic $I_z$ (BH) model] with parameter values estimated from voltage-clamp experiments; an HH-type model describing the membrane electrical behavior of an isopotential neuron with only two ionic currents—$I_h$ and a leak current [the reduced membrane (RM) model]; a model of the subthreshold frequency-response relationship of neurons produced by linearizing the RM model; a hybrid model, comprised of the BH model and a living neuron, produced with the RCC technique; and a simplified RM model used to examine the sensitivity of various aspects of resonance to parameter changes.

In the following sections, we describe the construction and use of these models. The first section gives the structure of the BH model and its parameter values as estimated from voltage-clamp experiments. In the next section, the BI1 model is used as a basis for deriving the RM model and its frequency response. The RM model is used to construct theoretical FRCS for three resonant neurons. These FRCS are compared with those measured experimentally in the same neurons. An excellent agreement between the theoretical and experimental FRCS will confirm that our original assumption that $I_z$ is sufficient to produce subthreshold resonance. The following section on electronic pharmacology describes the use of the RCC with the BH model to endow a nonresonant neuron with resonance. This allows us to study the effect of resonance on spike production without explicitly modeling the complex dynamics of action potential genesis. We then examine the dependence of certain features of resonance on the parameters of the simplified RM model. In the final section, we study two other voltage-dependent currents found in neocortical neurons and their effects on resonance.

**FIG. 3.** Demonstration of electronic expression of an artificial $I_z$ in a model cell and in an in vitro neuron using RCC technique. A: computer-generated $I_z$ causes slow sags and overshoots in voltage responses of a model cell ($500 \ M\Omega$ resistor in parallel with a 33-pF capacitor) to square current pulses. Current-voltage ($I-V$) relation of the model-cell/RCC system shows inward rectification induced by electronic expression of $I_z$ in passive model cell. B: electronic expression of $I_z$ in a neocortical neuron intensifies subthreshold inward rectification. Quasi-steady state $I-V$ plots in A and B are generated by injected current ramps (25 $pA/s$).
Construction of the BH model

We first construct a model of $I_H$ based on experimental observations in neocortical neurons. We found that $I_H$ often activated with two time constants; this also has been observed in neocortical neurons by others (Solomon and Nerbionne 1993b; Spain et al. 1987). Therefore, to describe $I_H$ activation, we assumed the BH model given by

$$I_H = g_H(p_pm_f + p_pm_s)(V - V_{Hr}) (8A)$$

and

$$\frac{dm}{dt} = \frac{m_a(V) - m_f}{\tau_f(V)} (8B)$$

and

$$\frac{dm}{dt} = \frac{m_a(V) - m_s}{\tau_s(V)} (8C)$$

where

$$m_a(V) = \left[ 1 + \exp\left( \frac{V - V_{Hr}}{k_m} \right) \right]^{-1} \tag{9}$$

Here, $g_H$ is the maximal conductance of $I_H$, $V_H$ is its reversal potential, $m_a$ is the steady state activation, $V_{Hr}$ is the membrane potential when $m_a = 0.5$, $k_m$ is a factor that controls the slope of the activation curve, and $r = 1$. The two time constants for $I_H$ activation are denoted $\tau_f$ and $\tau_s$ for the fast and slow time constants, respectively (Solomon and Nerbionne 1993b). We did not specify functional forms for $\tau_f(V)$ and $\tau_s(V)$; instead, at each membrane potential, we used experimentally measured values. In the BH model, the two components of $I_H$ activation share the same voltage dependence but require different activation variables, $m_f$ and $m_s$. The proportions of the conductance associated with the fast and slow components of $I_H$ were $p_f$ and $p_s$, respectively.

We now show how we estimated the values of the parameters in the BH model.

**Activation Kinetics.** In voltage-clamped neocortical neurons, an inward current that activated slowly in response to hyperpolarizations from holding potentials near threshold was unambiguously identified as $I_H$. Although it is unlikely that a complete space clamp was achieved in these geometrically complex neurons, $I_H$ activated as a graded function of voltage and its time course of activation did not show signs of an unclamped current.

To extract the time constants of $I_H$ activation, the time courses of currents evoked by hyperpolarizing steps from a holding potential near $-60$ mV (Fig. 4A) were fitted with a sum of exponential terms

$$I(t) = A_f + A_re^{-\tau_f + A_s}(1 - e^{-\tau_s}) + A_s(1 - e^{-\tau_s}) \tag{10}$$

In Eq. 10, the last two terms contain the fast and slow $I_H$ time constants and the asymptotic values of the fast and slow components of $I_H$ ($A_f$ and $A_s$). Both $\tau_f$ and $\tau_s$ were $>0.05$ s at membrane potentials more positive than $-100$ mV. Two other currents, $I_{nak}$ and $I_{K}$, contributed almost instantaneous components to the total current (cf. Scroggs et al. 1994). In Eq. 10, these currents are lumped into $A_f$. The term describing the capacitive transient in Eq. 10 (with parameters $A_r$ and $\tau_r$) was required to produce a reliable fit.

Figure 4B shows the voltage dependence of the $I_H$ time constants in eight regular-spiking (RS) neurons. These neurons were chosen for analysis because $I_H$ was large. Seven of the neurons required two time constants to describe $I_H$ activation over some part of its range. When the neurons were held near $-90$ mV, $\tau_f$ ranged from 0.09 to 0.24 s and $\tau_s$ ranged from 0.64 to 2.40 s (Table 1; Fig. 4B, $I$ and 2). Both time constants tended to decrease with hyperpolarization. In the single neuron where $I_H$ activation was characterized by a single time constant, its value was comparable with the lower range of $\tau_s$ in the other neurons (Fig. 4B2, $I$).

**Reversal Potential.** The reversal potential of $I_H$ was estimated by comparing the instantaneous and steady state $V - I$ relationships of neocortical neurons (Fig. 4C). Referring...
TABLE 1. Estimated parameter values for $I_H$ activation at 24–26°C

<table>
<thead>
<tr>
<th>Cell</th>
<th>$g_{m}$, nS</th>
<th>$V_{H2}$, mV</th>
<th>$k_{m}$, mV$^{-1}$</th>
<th>$V_{H}$, mV</th>
<th>$\tau_{r}$, s</th>
<th>$\tau_{s}$, s</th>
<th>$p_{r}$†</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>14.2</td>
<td>-71</td>
<td>8.6</td>
<td>-35</td>
<td>0.13</td>
<td>1.20</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>-81</td>
<td>8.2</td>
<td>-45</td>
<td>0.15</td>
<td>0.84</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>3.7</td>
<td>-72</td>
<td>6.6</td>
<td>-40</td>
<td>0.18</td>
<td>2.40</td>
<td>0.49</td>
</tr>
<tr>
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<td>-86</td>
<td>9.1</td>
<td>-41</td>
<td>0.17</td>
<td>1.29</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>7.2</td>
<td>-74</td>
<td>5.1</td>
<td>-39</td>
<td>0.24</td>
<td>1.73</td>
<td>0.60</td>
</tr>
<tr>
<td>6</td>
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<td>-82</td>
<td>10.3</td>
<td>-40</td>
<td>0.93</td>
<td>0.64</td>
<td>0.69</td>
</tr>
<tr>
<td>7</td>
<td>2.9</td>
<td>-92</td>
<td>4.6</td>
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</tr>
<tr>
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<td>-42</td>
<td>0.91</td>
<td>1.94</td>
<td>0.71</td>
</tr>
</tbody>
</table>

* Fitted time constants for activation of $I_H$ at $-90$ mV. † Proportion of $I_H$ associated with $\tau_r$ at $-90$ mV: $p_r = A_r(A_r + A_s)^{-1}$. ‡ Neuron with a single time constant for activation of $I_H$.

Construction and analysis of the RM model

The BH model formed the basis for a reduced model of membrane electrical behavior, called the RM model. This is an HH-type model of a neuron possessing only $I_H$ and $I_{leak}$. This model was most appropriate for describing the electrical behavior of RS neurons between $-80$ and $-65$ mV because other subthreshold voltage-dependent currents in these neurons are seldom active over this range of potentials (see Hutcheon et al. 1996).

The RM model therefore is given by Eqs. 8, A–C, and 9 together with

$$
c_m \frac{dV}{dt} = -g_{leak}(V - V_{leak}) - I_H + I_{nj} 
$$

where $g_{leak}$ is the conductance of the leak current and $V_{leak}$ is its reversal potential. We integrated these equations numerically using parameter values from Table 1 above and values of $g_{leak}$ and $c_m$ calculated from Table 1 of the companion paper (RS neurons) (Hutcheon et al. 1996). Figure 5 shows that the voltage responses to pulse or ZAP (Puil et

![Figure 5](image-url)
where $J = R_{\text{static}} + 2\pi f c m + g_{\text{H}}[1 + H_f + H_s]$ (13)

Thus the $\text{FRC}$ in Fig. 6B was measured at $-69 \text{ mV}$ but was added to the membrane potentials of both neurons. The neuron was added to the membrane potentials of both neurons. For three of the neurons listed in Table 1, we constructed theoretical FRCs after parameter adjustments to produce better fits. Dashed-dotted line in each plot is passive response that remains after contributions of $I_{\text{Li}}$ are removed from models. A: a neuron that did not require any adjustments to measured parameter values to produce a good fit between theoretical and observed FRCs. Unadjusted parameter values are $p_f = 8.2 \text{ nS, } c_m = 290 \text{ pF, } \tau_f = 0.189 \text{ s, } p_\text{H} = 0.4$. B: a neuron requiring, for a good fit, a 3-mV offset in membrane potential. Adjusted parameter values are $g_{\text{H}} = 3.9 \text{ nS, } c_m = 290 \text{ pF, } g_f = 3.7 \text{ nS, } \tau_f = 0.943 \text{ s, } \tau_\text{H} = 0.171 \text{ s, } p_f = 0.5$. C: a neuron requiring a 4-mV offset and a change in $p_f$ from 0.6 to 0.8. Adjusted parameters are $g_{\text{H}} = 1.7 \text{ nS, } c_m = 300 \text{ pF, } g_f = 14.2 \text{ nS, } \tau_f = 8.6 \text{ mV}^{-1}$. $V_{\text{H}} = 71 \text{ mV, } V_a = 35 \text{ mV, } \tau = 1.281 \text{ s, } \tau_\text{H} = 0.189 \text{ s, } p_f = 0.8$. In A, C, unadjusted parameter values for the RM model for individual neurons were gathered either from voltage-clamp experiments (parameters describing $I_{\text{H}}$) or from fits of voltage responses to small-amplitude current steps after $I_{\text{H}}$ was blocked by $3 \text{ mM Cs}^+$. (leak conductance and input capacitance). D: theoretical FRC for a cat sensorimotor neuron at $36 \text{ C}$ (dashed line). Parameter values were taken from a model by Spain et al. (1989): $g_{\text{H}} = 37 \text{ nS, } c_m = 370 \text{ pF, } g_f = 29 \text{ nS, } \tau_f = 7 \text{ mV}^{-1}$. $V_{\text{H}} = -78 \text{ mV, } V_a = -35 \text{ mV, } \tau = 0.319 \text{ s, } \tau_\text{H} = 0.038 \text{ s, } p_f = 0.2$. Note shift off $f$. Second, the relative weights of the fast and slow components of $I_{\text{H}}$ (i.e., $p_f$ and $p_\text{H}$) had to be changed to emphasize the slow component. The values of the measured parameters for the theoretical FRCs are given in the figure legends.
and the adjusted parameter values required to achieve a good fit are shown in each figure.

When the offset and weight adjustments were made, the resulting theoretical FRCs (dotted curves) fit the measured FRCs very well. This demonstrates that the elements of the RM model ($I_{\text{RH}}$, $I_{\text{leak}}$, and $c_s$) are sufficient to reproduce the major features of the FRCs of neocortical neurons. The predicted FRC when $g_{\text{RH}}$ is set to 0 in the model also is shown in Fig. 6 (dot-dash curve). Comparison of the FRCs with and without $I_{\text{RH}}$ indicates that $I_{\text{RH}}$ is the major voltage dependent current responsible for the resonant hump and the characteristic resonant frequency in neocortical neurons. In conjunction with the previous demonstration that pharmacologically blockade of $I_{\text{RH}}$ abolishes subthreshold resonance in these neurons (Hutcheon et al. 1996), this shows that $I_{\text{RH}}$ is necessary to produce resonance.

PREDICTED FREQUENCY RESPONSE AT PHYSIOLOGICAL TEMPERATURE. The parameter values we used in the RM model led to theoretical FRCs with resonant frequencies between 1 and 2 Hz near $-70$ mV. These values were derived from experiments at 24–26°C. However, Spain et al. (1987) have found that the activation time constants of $I_{\text{RH}}$ in cat sensorimotor neurons maintained in vitro at 36°C are 6–11 times faster than those in Fig. 4B. Their values for $g_{\text{leak}}$ and $g_{\text{RH}}$ per unit input capacitance also are larger. This suggests that $I_{\text{RH}}$ is highly temperature sensitive. In support of this, Solomon and Nerbonne (1993a,b) and Budde et al. (1994) give values for $\tau_s$ and $\tau_f$ in dissociated rat visual cortical neurons at 20–22°C that are comparable with those in Fig. 4B.

To investigate the possible values of the resonant frequency ($f_{\text{res}}$) at physiological temperatures, we used the parameter values reported by Spain et al. (1987) for their model of a sensorimotor neuron with $I_{\text{RH}}$ at 36°C. The resulting theoretical FRC for $-70$ mV, plotted in Fig. 6D, shows that $f_{\text{res}}$ is near 10 Hz under these conditions. Over potentials from $-60$ to $-80$ mV, $f_{\text{res}}$ and varied from 5 to 15 Hz. Thus resonant frequencies generated by $I_{\text{RH}}$ at 24°C may be an order of magnitude lower than at physiological temperatures.

In vitro modeling of $I_{\text{RH}}$ using electronic pharmacology

In a previous set of experiments, we found that resonant neurons preferred to fire action potentials when stimulated with oscillatory current inputs near $f_{\text{res}}$. Stimulation with oscillatory currents at nonresonant frequencies produced smaller subthreshold voltage responses and fewer action potentials. Nonresonant neurons did not have a resonant frequency or exhibit a frequency-selective coupling to the firing of action potentials. Using the RCC, we were able to replicate the frequency preference of resonant neurons by coupling nonresonant neurons to a mathematical model of $I_{\text{RH}}$.

As explained in METHODS, the RCC injects a computer-generated current corresponding to a voltage-dependent conductance into neurons. This current was used either to antagonize an endogenous $I_{\text{RH}}$ or to express an artificial $I_{\text{RH}}$ in neurons. This allowed us to study the interaction of the modeled $I_{\text{RH}}$ with the spike firing mechanism of neurons. The mathematical model of $I_{\text{RH}}$ that we used for the RCC was similar to the BH model (Eq. 8) except that, for simplicity, we assumed a single activation time constant for $I_{\text{RH}}$. Therefore, the model is given by

$$I_{\text{RH}} = -g_{\text{RH}}(V - V_{\text{RH}})$$

Equation 5 for the activation variable, $m$. The functional forms for the steady state activation ($m_s$) and activation time constant ($\tau_m$) are given by Eq. 9 and

$$\tau_m = \frac{2m_s}{\exp\left(\frac{V - V_{\text{RH}}}{k_m}\right) + \exp\left(-\frac{V - V_{\text{RH}}}{k_m}\right)}$$

respectively. Here, $\tau_0$ is the value of the time constant at $V_{\text{RH}}$.

ELECTRONIC ANTAGONISM OF $I_{\text{RH}}$. As a check on the fidelity of this model, we first used the EP-method to antagonize the endogenous $I_{\text{RH}}$ of a neuron. Figure 7 shows the voltage responses for a neuron before and after antagonizing the endogenous $I_{\text{RH}}$ by injection of a negative copy of $I_{\text{RH}}$ generated by a computer model (see METHODS). In this case, the electronic antagonism successfully removed the sags and rebounds characteristic of voltage responses to hyperpolarizing current inputs in neurons with $I_{\text{RH}}$. In many neurons, however, annihilation of the sags did not result in the total cancellation of the rebounds. This probably reflected the existence of two activation time constants for $I_{\text{RH}}$ in most neurons (see Fig. 4).

For each neuron, the electronic antagonism required fine tuning of the model parameters. Because different parameter values were required for each neuron, we initially used parameter values that seemed reasonable, based on prior experience. We then changed the parameter values one by one to produce an effective antagonism. Some of the parameters had a wider range of variation than others. The most variable parameters were adjusted first which produced the following order: $g_{\text{RH}}$, $\tau_0$, $V_{\text{RH}}$, $k_m$, and $V_{\text{RH}}$. Often, only $g_{\text{RH}}$ and $\tau_0$ required adjustment. To show that this procedure results in a well-defined set of parameter values, Fig. 7 shows the result of intentionally adjusting $g_{\text{RH}}$ and $\tau_m$ to incorrect values. Although the parameter values used here were within the range of variation found in other neocortical neurons (see Table 1), they were clearly unsuitable for the neuron shown here.

ELECTRONIC EXPRESSION OF $I_{\text{RH}}$. Electronic expression of an artificial $I_{\text{RH}}$ in a neuron with little or no endogenous $I_{\text{RH}}$ produced a sag in the voltage response to a hyperpolarizing current pulse. The FRC of the neuron also acquired a resonant hump near 2 Hz (Fig. 8A). This shows that a current input in a neuron with little or no endogenous $I_{\text{RH}}$ can cause resonance in neocortical neurons.

We directly demonstrated that the resonance due to $I_{\text{RH}}$ can couple to the spike firing mechanism to produce frequency-selective firing. To do this, we compared the frequency-coupling of spikes evoked by a ZAP current stimulus in neurons before and after expressing an artificial $I_{\text{RH}}$ (ZAP voltage traces, Fig. 8A). For the case of an artificial $I_{\text{RH}}$, action potentials fired preferentially as the ZAP input swept through frequencies near $f_{\text{res}}$. A similar coupling of resonance to frequency-selective spike firing was seen in neurons with an endogenous $I_{\text{RH}}$ (cf. Fig. 1C) (Hutcheon et al. 1996). We also used current inputs consisting of single-frequency sine waves to evoke firing in a neuron with an electronically expressed $I_{\text{RH}}$ (Fig. 8B). Firing was associated preferentially
FIG. 7. Electronic antagonism of \( I_H \) using RCC in an in vitro neuron. Under control conditions (left), sags in voltage responses to hyperpolarizing current pulses (arrowhead) indicate presence of \( I_H \). Sags are eliminated totally and resting potential of the neuron hyperpolarized (middle) by electronically antagonizing \( I_H \) in neuron. To demonstrate sensitivity of process, arrows (right) indicate voltage responses to a hyperpolarizing pulse when values of \( \tau_H \) and did not match properties of endogenous \( I_H \). Parameters used for this cell are \( V_H = -40 \text{ mV} \), \( V_{H2} = -85 \text{ mV} \), \( \tau_H = 500 \text{ ms} \), and \( k_m = 9 \).

with the input frequency nearest the resonant frequency, and individual spikes were phase-locked to the sinusoidal voltage response. Note, however, that the spikes and the sinusoidal voltage responses were not frequency-locked.

Construction and analysis of the simplified RM model of subthreshold resonance

In this section, we examine the dependence of certain features of resonance on the parameters of the RM model. To facilitate this, we used a version of the RM model that has only a single time constant for \( I_H \) activation. This is called the simplified RM model. The expression for the impedance of the simplified RM model is

\[
Z(f, V) = \frac{1}{j \omega_c + 2\pi f c_m + g_H[1 + H]} \tag{18}
\]

where

\[
H = \frac{g(V)}{1 + 2\pi f \tau_H} \tag{19}
\]

In an earlier section, we used Eq. 13 for the impedance of the RM model to find theoretical FRCs for individual neurons after we had specified fully their parameter values by voltage-clamp experiments. There, the FRC was defined as the magnitude of the impedance, as a function of frequency, for a given voltage. Here, we take the magnitude of the impedance of the simplified RM model (Eq. 18) and consider it more generally as a frequency-response surface, i.e., a function of \( f \) and \( V \). Isopotential cuts through the surface yield the FRCs. The parameter values of the simplified RM model are not tied to those of particular neurons; instead, we use a set of parameters typical for resonant RS neurons. These values then are varied systematically to illustrate how they affect various features of resonance (containing \( I_H \)).

Figure 9A shows the frequency-response surface of the simplified RM model for a set of parameter values chosen as representative of the neocortical neurons that we examined (see legend) and where \( \tau_H \) is assumed to be voltage independent. The corresponding FRCs are shown in Fig. 9B.

At membrane potentials above -50 mV, where \( I_H \) is not activated, the form of the frequency-response surface is solely due to the passive characteristics of the model, i.e., \( I_{\text{leak}} \) and \( c_m \). Thus in Fig. 9B, the FRC at 0 mV has the form of a lowpass filter with a cutoff frequency (in Hz) of \( f_{\text{c}} = \frac{2\pi \tau_m}{(2\pi f_m)^{-1}} \) (arrow). At membrane potentials more negative than -50 mV, resonance is apparent as a ridge, accompanied by a generalized low-frequency attenuation in the frequency-response surface (Fig. 9A). This also can be seen in the FRCs (Fig. 9B) where the resonance appears as a hump with a peak at \( f_{\text{c}} \). Comparing the low- and high-frequency ends of the FRCs at hyperpolarized potentials with the FRC at 0 mV, it is evident that the resonant hump forms because of a low-frequency attenuation that is enhanced by hyperpolarization.

Figure 9C shows how values of \( Q \) and \( f_{\text{c}} \) change with membrane voltage. The \( Q \) value is the ratio of the impedance magnitude at \( f = f_{\text{c}} \) to the impedance magnitude at \( f = 0 \) Hz. Note that \( f_{\text{c}} \) is voltage dependent even though we have made \( \tau_H \) voltage independent. Resonance first appears near -44 mV. At these potentials, however, the \( Q \) value is small (\( Q \)-value < 1.05) and \( f_{\text{c}} \) is near 0. The \( Q \) value first becomes significant (>1.05) at -60 mV; at this point the value of \( f_{\text{c}} \) is near 1.0 Hz. The \( Q \) value then climbs to a peak value of 1.8 at -80 mV whereas \( f_{\text{c}} \) peaks at a value of 2.1 Hz near -84 mV. Finally, both the \( Q \) value and \( f_{\text{c}} \) decline with further hyperpolarization. Note that, between -60 and -80 mV, the relationship between \( f_{\text{c}} \) and voltage is almost linear. These features qualitatively match the typical features of resonance in neocortical neurons (Hutcheon et al. 1996) (cf. Fig. 3B). Note that all of the voltage-dependent changes in the FRCs and the frequency-response surface occur at frequencies <20 Hz. This also was observed in neocortical neurons (Hutcheon et al. 1996).

EQUIVALENT CIRCUITS FOR ANALYZING THE SIMPLIFIED RM MODEL.

The simplified RM model can be represented by an equivalent circuit consisting of three parallel impedance branches, corresponding to the passive leak conductance, the frequency-dependent contribution of the membrane capacitance, and the frequency and voltage-dependent impedance
due to $I_H$ (see Fig. 10A1). The impedance of the circuit is determined from
\[
\frac{1}{Z} = \frac{1}{Z_{\text{leak}}} + \frac{1}{Z_{\text{cap}}} + \frac{1}{Z_H}
\]  
(20)

where
\[
Z_{\text{leak}} = \frac{1}{g_{\text{leak}}}, \quad Z_{\text{cap}}(f) = \frac{1}{2\pi ifc_m}, \quad \text{and} \quad Z_H(f, V) = \frac{1}{g_H[1 + H]}
\]

Reassembling the components of this equivalent circuit in different configurations clarifies the origin of the resonant hump in the FRC, cf. Fig. 10B for $V = -70$ mV. Consider the equivalent circuit composed of $Z_{\text{leak}}$ and $Z_{\text{cap}}$ in parallel (Fig. 10A2) with impedance given by $[1/\bar{Z}_{\text{leak}} + 1/\bar{Z}_{\text{cap}}]^{-1}$. The impedance magnitude is shown in Fig. 10B (dashed curve). This circuit attenuates high-frequency inputs; we call it the high-frequency attenuator circuit. Its frequency dependence is characterized by the cutoff frequency, $f_m = (2\pi \tau_m)^{-1}$ (labeled arrow, Fig. 10B).

Similarly, an equivalent circuit composed of $Z_{\text{leak}}$ and $Z_H$ in parallel has impedance $[1/\bar{Z}_{\text{leak}} + 1/\bar{Z}_H]^{-1}$. The impedance magnitude is plotted in Fig. 10B (dotted curve), which shows that this circuit is a low-frequency attenuator characterized by the cutoff frequency $f_H = (2\pi \tau_H)^{-1}$ (labeled arrow, Fig. 10B). At high frequencies, the impedance magnitude asymptotically approaches $1/|\Gamma_\alpha|$ where $\Gamma_\alpha(V) = g_{\text{leak}} + g_H$ is the chord conductance of the simplified RM model. At low frequencies, the im-
pedance magnitude approaches \(1/\Gamma_0\) where \(\Gamma_0(V) = g_{\text{out}} + g_H(1 + \xi)\) is the slope conductance of the simplified RM model.

The resonant hump of the total impedance magnitude, \(|Z|\), arises in the relatively unattenuated region between the high- and low-frequency attenuations (solid curve, Fig. 10B). On the left and right sides of the resonant hump, the FRC closely follows the curve associated with the low-frequency attenuator circuit and the high-frequency attenuator circuit, respectively. Thus the impedance magnitude curves of the high- and low-frequency attenuator circuits delineate the FRC of the model. Note that in Fig. 10B, \(f_{\text{res}}\) (labeled arrow) lies between \(f_u\) and \(f_h\).

EQUATION FOR THE RESONANT FREQUENCY. From Eq. 18 for the impedance of the simplified RM model, it is possible to derive a functional form for \(f_{\text{res}}\). This is done by setting \(\partial |Z|/\partial f = 0\) and solving for \(f\), yielding

\[
f_{\text{res}} = \frac{\sqrt{f_u(f_u - f_h)}}{\sqrt{g_H(1 + \xi)}}
\]

(21)

where

\[
\xi = \frac{g_{\text{out}}(\Gamma_0 + \Gamma_u)}{(\Gamma_{\text{out}})^2}.
\]

Note that \(\xi\) depends on voltage because all of its terms except \(g_{\text{out}}\) are voltage dependent. Equation 21 shows that \(f_{\text{res}}\) is determined by the value of \(f_u\) and the difference between \(f_u\) and a scaled version of \(f_H\). This is consistent with the description, developed above, that resonance arises in the gap between the regions of high- and low-frequency attenuation controlled by \(f_u\) and \(f_h\), respectively. In Fig. 9C, we plot \(f_{\text{res}}\) (solid curve) together with \(f_u\) and \(f_h\) (labeled dashed curves). Note that \(f_{\text{res}}\) lies between \(f_u\) and \(f_h\) at most membrane potentials and that it can achieve values more than five times greater than \(f_u\). In Fig. 9C, \(f_{\text{res}}\) falls outside the limits established by \(f_u\) and \(f_h\) only at the boundaries of the resonant region, where resonance is weak (\(Q < 1.05\)).

The exact form of Eq. 21 provides a condition for the existence of resonance

\[
f_{\text{res}} < \frac{f_u}{f_h}\left(\frac{g_{\text{out}}}{\Gamma_{\text{out}}} + \frac{g_H}{\Gamma_u} - 1\right)
\]

(22)

When this condition is violated, i.e., when \(f_h\) is too large compared with \(f_u\), the region of low-frequency attenuation due to \(f_u\) overlaps with the region of high-frequency attenuation due to the capacitance to such an extent that there is no possibility for a resonant hump to arise.

EFFECTS OF PARAMETER VARIATIONS. We examined how varying some of the parameters of the simplified RM model affected the frequency response. We concentrated on altering the parameters that are likely to vary either from neuron to neuron or within a neuron.

First, consider the properties of \(I_H\) that may vary from neuron to neuron and the effects of differences in these properties. For instance, studies have shown that the H-conductance density can differ substantially in cortical neurons (see Table 1, this paper; Solomon et al. 1993). In Fig. 11A, we replicate such a difference via a twofold increase or decrease in the value of \(g_H\) (i.e., from 6 to 12 nS or 6 to 3 nS). The increase in \(g_H\), while leaving all other parameters unchanged, modified the FRC (at \(-70\) mV) by decreasing its peak amplitude by a factor of 0.7 and increasing the values of both \(f_{\text{res}}\) and \(Q\) by a factor of 1.3. The decrease in \(g_H\) resulted in changes in the opposite direction. These effects may be understood by referring to the analysis of the frequency response introduced above. Thus changes in \(g_H\) produce vertical shifts of the low-frequency attenuator curve in Fig. 10B without affecting the high-frequency attenuator curve. The FRC for the simplified RM model, delineated by these curves, changes accordingly.
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We also examined the effects of altering the total capacitance \( c_m \) while holding the specific conductances \( g_m \) and \( g_{\text{leak}} \) constant (Fig. 11B). Doubling \( c_m \) from 150 to 300 pF is equivalent to doubling the size of the modeled neuron without affecting the current densities. This change did not alter the values of \( f_{\text{ret}} \) and \( Q \); however, the overall amplitude of the FRC diminished by a factor of 0.5. Decreasing \( c_m \) enlarged the FRC by a factor of 2. Thus we would not expect differences in size among cortical neurons of the same type to alter their resonant characteristics. The resulting changes in input resistance, however, are reflected precisely in the peak values of the FRCs.

The activation time constants for \( I_A \) can vary widely among neocortical neurons (see Fig. 4B, 1 and 2) (Solomon and Nerbonne 1993b). We therefore determined the effects of altering \( f_H \) in the simplified RM model. Changes in \( f_H \) affect the bandwidth of the resonant hump and the values of \( Q \) and \( f_{\text{ret}} \) as shown in Fig. 11C. Here, \( f_H \) ranges from 32 Hz (corresponding to \( \tau_m = 0.005 \) s) to 0.032 Hz (corresponding to \( \tau_m = 5 \) s) whereas the value of \( f_{\text{ret}} \) is always 4.2 Hz (corresponding to \( \tau_m = 0.037 \) s). This is a larger range of \( f_H \) variation than actually encountered in our neurons (range: fast activation, \( f_H = 0.4-3.1 \) Hz; slow activation, \( f_H = 0.05-0.23 \) Hz). However, consideration of this expanded range clarifies the relationship between \( f_H \), \( f_{\text{ret}} \), and resonance (see below). Decreasing \( f_H \) from the standard value of 0.32 Hz (\( \tau_m = 0.5 \) s) to 0.032 Hz (\( i_H = 5 \) s), broadened the resonant hump and shifted \( f_{\text{ret}} \) to lower values but did not affect the value of \( Q \). Referring to Fig. 10B, the decrease in \( f_H \) corresponds to a leftward shift of the low-frequency attenuator curve. This widens the frequency gap between the regions of high- and low-frequency attenuation and increases the resonant bandwidth. Conversely, an increase in \( f_H \) produces a rightward shift of the low-frequency attenuator curve, narrowing the gap, diminishing the bandwidth and amplitude of the resonant hump, and increasing \( f_{\text{ret}} \). Finally, resonance disappears when \( f_H \) is large enough to violate the condition of Eq. 23. This situation is demonstrated for \( f_H = 32 \) Hz in Fig. 11C and corresponds to a complete overlap between the regions of high- and low-frequency attenuation in the high- and low-frequency attenuator curves of Fig. 10B.

Now consider changes in membrane properties that may

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**Fig. 11.** Effects of changes in parameter values of simplified RM model. A–D: frequency-response curves at \(-70\) mV for simplified RM model. Each figure shows FRCs resulting from changes in 1 parameter value. Keys (top right) show adjusted values. Solid curve in each figure shows the FRC for standard parameter values specified in Fig. 9.
occur transiently within a neuron. For example, multiple neuromodulatory systems may modify $g_{\text{leak}}$ in cortical neurons (McCormick 1992). Compared with changes in $g_{\text{leak}}$, changes in $g_H$ affected the frequency response in a more complex manner (Fig. 11D). Doubling $g_{\text{leak}}$ from 4 to 8 nS diminished the peak amplitude of the FRC by a factor of 0.6 and increased $f_{\text{res}}$ by a factor of 1.1. These effects were in the same direction as those due to a twofold increase in $g_H$. On the other hand, halving $g_{\text{leak}}$ raised the $Q$ value by a factor of 1.3, opposite to the effects of an equivalent change in $g_H$. The mixed effects of changes in $g_{\text{leak}}$ may be explained by noting that doubling $g_{\text{leak}}$ doubles the amount of leakage current available to shunt input currents as well as the value of $f_{\text{res}}$. The increased shunt accounts for the reduction in the $Q$ value, whereas the change in $f_{\text{res}}$ increases $f_{\text{res}}$ as seen from Eq. 21. Graphically, the increase in $f_{\text{res}}$ is due to a rightward shift in the curve for the high-frequency attenuator component of the impedance (see Fig. 10B).

Finally, the effect of a shift in the half-activation point ($V_{1/2}$) of the steady state activation curve for $I_H$ is to produce a corresponding shift in the voltage dependence of all the properties of the frequency response. Graphically, this corresponds to a shift of the frequency-response surface of Fig. 9A. This is because the membrane voltage, $V$, always appears in the combination $V - V_{1/2}$ in the expression for the impedance of the simplified RM model (Eq. 18). Several neurotransmitters shift $V_{1/2}$ in central neurons (Banks et al. 1993; McCormick and Pape 1990). One consequence of this effect would be a shift of the voltage dependence of resonance while leaving other voltage-dependent properties unchanged.

**Amplification and attenuation of resonance by $I_{\text{IR}}$ and $I_{\text{NaP}}$.**

We previously found that the activation range of $I_H$ in neocortical neurons sometimes overlaps with the activation ranges of two other rectifying, noninactivating currents identified as $I_{\text{IR}}$ and $I_{\text{NaP}}$ (Hutchison et al. 1996). With the aid of pharmacological blockers, we showed that these currents interact with $I_H$ to modify the FRCs of resonant neurons. Coactivation of $I_{\text{IR}}$ and $I_H$ caused a flattening of the resonant hump and an overall reduction in the peak magnitude of the FRC whereas coactivation of $I_{\text{NaP}}$ and $I_H$ sharpened the resonant hump and increased the peak magnitude of the FRC (Hutchison et al. 1996). For these reasons, $I_{\text{IR}}$ and $I_{\text{NaP}}$ are called attenuating and amplifying currents, respectively. In this section, we show that these actions are consistent with the changes produced by introducing $I_{\text{IR}}$ and $I_{\text{NaP}}$ into the simplified RM model.

Extending the simplified RM model to include currents that approximated $I_{\text{IR}}$ and $I_{\text{NaP}}$ resulted in attenuation and amplification of the resonance. We used the same HH-type formalism for $I_{\text{IR}}$ and $I_{\text{NaP}}$ as for $I_H$, except that we assumed both currents activated instantaneously. This assumption is a good approximation because the activation of both currents is much faster ($I_{\text{NaP}}$ Alzheimer 1993; $I_{\text{IR}}$, Sutor and Hablitz 1993) than either the average $\tau_m$ (0.4 s; Table 1) (Hutchison et al. 1996) or the activation time constants of $I_H$ measured in our neurons. The parameters characterizing the voltage dependence of the currents were derived from published observations in central neurons ($I_{\text{NaP}}$) (Sah et al. 1990) or our experimental observations ($I_{\text{IR}}$).

**DISCUSSION.**

An important result of our experimental and modeling studies using the FRCs is that the inwardly rectifying cation current ($I_H$) underlies the observed 1–2 Hz resonance in neocortical neurons. We found that $I_H$ is the major determinant of resonance at membrane potentials between −80 and −65 mV. These studies show that the voltage dependence and kinetics of $I_H$, together with the leakage current and capacitive properties, are sufficient to account for the resonance. This complements our previous pharmacological demonstration (Hutchison et al. 1996) that $I_H$ is necessary for resonance. In addition to changes in the properties of $I_H$, changes in other voltage-dependent currents ($I_{\text{NaP}}, I_{\text{IR}}$), and leakage and capacitive currents may modify the resonance. Nevertheless, a simple model of $I_H$, using parameter values taken directly from voltage-clamp measurements in individ-
ual neurons, replicated the FRCs in the same neurons. A functional consequence is that the neurons fire preferentially when stimulated with oscillatory currents near their resonant frequency (frequency-selective firing). By coupling the model of \( I_h \) to living neurons using a RCC, we obtained an independent confirmation of the role of \( I_h \) in producing resonance and frequency-selective firing.

**Electronic pharmacology**

In the in vitro modelling studies, we used the RCC to cancel an endogenous \( I_h \) (electronic antagonism) and to introduce an artificial \( I_h \) (electronic expression) when an endogenous \( I_h \) was absent in a living neuron. Previous pharmacological investigations often relied on the use of selective antagonists that additionally may block other currents (e.g., Cs\(^+\) blocks \( I_h \) as well as \( I_{IR} \) and \( I_{leak} \)). Also, current pulse injections that hyperpolarize a neuron into the usual activation range for \( I_{IR} \) may elicit a contribution of \( I_{IR} \) to the sagging voltage and rebound responses (e.g., Fig. 7, control). The selective electronic antagonism of \( I_h \) using a mathematical model completely eliminated the voltage sags, demonstrating that \( I_{IR} \) did not make a significant contribution in such neurons (cf. Figure 7). This represents the first use of an in vitro modelling technique to antagonize a specific endogenous current, \( I_h \). The electronic expression of an \( I_h \) model introduced resonance and frequency-selective firing into the voltage responses of neurons without an \( I_h \). This finding also allows us to draw specific conclusions regarding the relationship among \( I_h \), resonance, and frequency-selective firing in neocortical neurons. Because the RCC reproduces only the electrical, and not chemical nature of the endogenous \( I_h \), only the electrical properties are essential for resonance. Neither resonance nor frequency-selective firing can depend on the accumulation of internal Na\(^+\) ions that enter through H channels. Because the conductance created by the RCC technique is restricted to the site of current injection through the electrode, the production of resonance and consequent changes in firing pattern also does not require a distributed \( I_h \) conductance.

**Resonance**

How does \( I_h \) produce resonance? Our view is that a combination of low-frequency attenuation due to \( I_h \) and high-frequency attenuation due to the capacitive properties of the membrane shapes the profile of the FRCs for neocortical neurons. Under the proper conditions, a resonant hump can arise at intermediate frequencies.

The current, \( I_h \), reduces voltage transients, including those evoked by oscillating currents, by opposing low-frequency changes in the membrane voltage. Hyperpolarization activates \( I_h \), resulting in a slowly developing inward current that partially counteracts the hyperpolarization. Depolarization deactivates \( I_h \), reducing the inward current, which then partially reverses the depolarization. These reductions are largest at stimulation frequencies with periods that are long compared with the time constants of \( I_h \). The net effect is an attenuation at low frequencies (Fig. 10B) where the activation level of \( I_h \) can follow the current input. At higher stimulation frequencies, the ability of \( I_h \) to keep up with the input is overwhelmed, thereby reducing the attenuation. Thus \( I_h \) in combination with the high-frequency attenuation due to the capacitance can produce a resonance (resonant current or "resonator").

**Amplification**

The persistent Na\(^+\) current (\( I_{Nap} \)) causes a voltage-dependent amplification of the resonant hump in neurons (Gutfreund et al. 1995; Hutcheon et al. 1996; Str"ohmann et al. 1994). In contrast to the currents that oppose voltage changes as discussed above, \( I_{Nap} \) is representative of a class of voltage-dependent currents that potentiate voltage changes. This is revealed readily from the theoretical models. Depolarization activates \( I_{Nap} \), increasing the inward current, which further depolarizes the membrane potential. Hyperpolarization turns off \( I_{Nap} \), decreasing the inward current, which leads to further hyperpolarization. This positive feedback mechanism explains the anomalous voltage-dependence of the excitatory postsynaptic potential magnitude in neocortical neurons (Deisz et al. 1991; Stafstrom et al. 1985). This is similar to the regenerative mechanism that causes the rising phase of action potentials (Hodgkin and Huxley 1952).

In the context of the frequency-domain analysis, the potentiation of voltage changes by \( I_{Nap} \) increases the impedance magnitude. Thus if \( I_{Nap} \) replaces \( I_h \) in the circuit of Fig. 10A, the corresponding impedance magnitude has
cies of spontaneous oscillations are related to the resonant frequency. This increase occurs over a large range of frequencies because \( \Delta_{\text{Nap}} \) activates rapidly in neocortical neurons (<0.004 s, Stafstrom et al. 1985; <0.001 s, Alzheimer et al. 1993). Therefore, the effect of \( \Delta_{\text{Nap}} \) appears as a broadband amplification of the FRC. This amplification intensifies the resonance, leading to significantly higher \( Q \) values, cf. experimentally observed \( Q \) values for the \( I_{\text{f}} \) resonance (Hutchison et al. 1996, Table 2).

In neocortical neurons, other currents that potentiate voltage changes through regenerative mechanisms may amplify the resonance. These include a high-voltage-activated Ca\(^{2+}\)-current (Brown et al. 1993; Puil et al. 1994), a N-methyl-D-aspartate (NMDA)-activated current (Moore et al. 1993), and a subthreshold, inwardly rectifying current that is sensitive to blockade by Co\(^{2+}\) (Sutor and Ziegglansberger 1987). In cerebellar Purkinje cells, an amplification of low-frequency responses is associated with regenerative, noninactivating Na\(^+\) or Ca\(^{2+}\) currents (Jahnsen and Karnup 1994), possibly amplifiers of resonance.

A single current may produce resonance as well as its amplification. This was shown, for example, in a model of thalamic neurons where resonance resulted from an interaction of the kinetic properties of the low-threshold Ca\(^{2+}\) current (\( \Delta_{\text{T}} \)) (Hutchison et al. 1994). The fast \( \Delta_{\text{T}} \) activation amplified a resonance that is controlled mainly by \( \Delta_{\text{T}} \) inactivation. This model was capable of spontaneous oscillations.

Amplified resonance and spontaneous oscillations

Amplified resonances may initiate spontaneous oscillations of the membrane potential in various cortical (Alonso and Klink 1993; Amitai 1994; Gutfreund et al. 1995; Jahnsen and Karnup 1994; Llinás et al. 1991; Nuñez et al. 1992), thalamic (Hutchison et al. 1994), and spinal neurons (Moore et al. 1993). An \( I_{\text{f}} \) alone cannot maintain small amplitude, sustained oscillations because a system with only a resonant current component \( g_{\text{leak}} \) always has stable steady states. In some cortical neurons, the concerted actions of a depolarization-activated K\(^+\) current and \( \Delta_{\text{Nap}} \) generate resonance (Gutfreund et al. 1995) and spontaneous oscillations (Klink and Alonso 1993). In spinal neurons, spontaneous oscillations also occur when an NMDA current amplifies a K\(^+\) resonance (Moore et al. 1993).

Resonance and spontaneous oscillations are mechanisms that can endow neurons with characteristic frequencies. For the above cases, modeling studies suggest that the frequencies of spontaneous oscillations are related to the resonant frequency (Gutfreund et al. 1995; Hutchison et al. 1994; Moore et al. 1993; Wang 1993). A finding from our modeling studies is that the \( I_{\text{f}} \)-resonance can have a \( f_{\text{res}} \) more than five times greater than \( f_{\text{f}} \). This suggests that the K\(^+\) currents that generate the resonance will have slow activation kinetics. Thus in entorhinal cortical neurons, the slowly activating K\(^+\) current may support 5–20 Hz spontaneous oscillations (Alonso and Klink 1993), as confirmed by the model of Gutfreund et al. (1995). In the case of spontaneously oscillating neurons, the preference for a characteristic frequency is overt whereas, in resonant neurons, the frequency preference is latent and only revealed in the presence of oscillatory inputs.

Functional modulation of resonance

Both \( I_{\text{f}} \) and resonance in neocortical neurons may be subject to neuromodulation. In some central neurons, for example, an elevation of internal adenosine 3’,5’-cyclic monophosphate levels can shift the activation curve of \( I_{\text{f}} \) along the voltage-axis to more depolarized levels (Banks et al. 1993; McCormick and Pape 1990). Our theoretical results show that this shift would change the voltage dependence of \( I_{\text{f}} \) resonance, specifically moving the peak values of \( Q \) and \( f_{\text{res}} \) along the membrane potential axis closer to the firing threshold. As a result, the coupling of firing to oscillatory inputs may occur over a widened frequency band (Hutchison et al. 1996).

Modulation of the maximal H conductance (\( g_{\text{H}} \)) and \( g_{\text{leak}} \) may affect the resonant behavior, as demonstrated with the simplified RM model. An increase in \( g_{\text{H}} \) attenuated the FRC and increased its \( Q \) value and \( f_{\text{res}} \) (cf. Fig. 11A). Indeed, \( g_{\text{H}} \) depends on age and projection target in rat neocortex (Kasper et al. 1994; Mason and Larkman 1990; Solomon et al. 1993) and may depend on internal [Ca\(^{2+}\)] in cat neocortex (Schwindt et al. 1992). Also we showed that a decrease in \( g_{\text{leak}} \) amplified the resonant hump, increased its \( Q \) value, and decreased \( f_{\text{res}} \) (cf. Fig. 11D). A change in \( g_{\text{leak}} \) affects \( f_{\text{f}} \), which determines whether a current like \( I_{\text{f}} \) is a resonator or an attenuator of the frequency response. This suggests a novel mode of neuromodulation in the cortex. Figure 13 shows that, under special conditions (note normalized impedance scales), a shift in \( f_{\text{f}} \) due to an increase in \( g_{\text{leak}} \) may expose a resonant hump. Because a repertoire of possible resonator currents with various kinetic properties exist in neocortical neurons (see above), changes in \( g_{\text{leak}} \) associated with different brain states may produce distinct resonances. During desynchronized states of the electroencephalogram (EEG), changes in the “effective” \( g_{\text{leak}} \) (input conductance) could greatly increase the range for the expression of resonant humps.
nance in neocortical neurons. An example is a neuron subjected to a sustained, random synaptic barrage, producing a summation of conductances (Berman et al. 1991). In such cases, the "effective" membrane time constant can be an order of magnitude smaller than $\tau_w$, which results in higher $f_{res}$.

**SIGNIFICANCE**

Neocortical neurons with $I_H$ are capable of responding to specific frequencies in the rhythmic activities of central neural networks. Based on a previous survey of neurons under in vitro conditions at 24–26°C (Hutcheon et al. 1996) and the estimated value of $f_{res}$ at physiological temperatures (see Fig. 6D), we predict that many neocortical neurons will respond in a voltage-dependent manner to frequencies between 5 and 15 Hz. This tuning is within the frequency band of neural rhythms and stabilize their frequencies.

This point of view extends the notion of the intrinsic rhythmicity of neurons, as implicated in the coordination of rhythmic activity in central neural networks (Llinàs 1988). Resonance may have importance in situations where neurons in a brain area receive pacemaker activity from a different region. In such cases, AC analysis techniques will provide an efficient method for investigating the mechanisms and contributions of individual neurons to network rhythmicity.

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