Modeling State-Dependent Inactivation of Membrane Currents

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ABSTRACT Inactivation of many ion channels occurs through largely voltage-independent transitions to an inactivated state from the open state or from other states in the pathway leading to opening of the channel. Because this form of inactivation is state-dependent rather than voltage-dependent, it cannot be described by the standard Hodgkin-Huxley formalism used in virtually all modeling studies of neuronal behavior. Using two examples, cumulative inactivation of the Kv3 potassium channel and inactivation of the fast sodium channel, we extend the standard formalism for modeling macroscopic membrane currents to account for state-dependent inactivation. Our results provide an accurate description of cumulative inactivation of the Kv3 channel, new insight into inactivation of the sodium channel, and a general framework for modeling macroscopic currents when state-dependent processes are involved. In a model neuron, the macroscopic Kv3 current produces a novel short-term memory effect and firing delays similar to those seen in hippocampal neurons.

INTRODUCTION

The general approach developed by Hodgkin and Huxley (1952) over 40 years ago to describe sodium and potassium currents in the squid giant axon has been successfully applied to many other membrane currents and now forms the basis for virtually all modeling studies of neuronal behavior. However, further research has clearly demonstrated that the behavior of membrane channels is often inconsistent with the general assumptions of the Hodgkin-Huxley formalism, and more elaborate microscopic descriptions have been developed (Armstrong and Bezanilla, 1977; Bean, 1981; Aldrich et al., 1983; Hoshi and Aldrich, 1988; DeCoursey, 1990; Zagotta and Aldrich, 1990; for a general overview, see Hille, 1992). Although important for understanding how channels operate, these studies have had only limited impact on macroscopic modeling because of their complexity, and because deviations from Hodgkin-Huxley behavior in macroscopic currents have tended to be quite small.

The results on cumulative inactivation of the Kv3 potassium channel reported in the companion manuscript (Marom and Levitan), and other studies of cumulative inactivation (DeCoursey, 1990; Aldrich, 1981; Marom et al., 1993), led us to reassess this situation. Cumulative inactivation is a significant phenomenon, with clear biological relevance, that cannot be modeled using the standard formalism. Within the Hodgkin-Huxley approach, inactivation is purely voltage-dependent and, in particular, is state-independent. As discussed in Marom and Levitan (companion manuscript), cumulative inactivation of the Kv3 channel appears to be state-dependent and voltage-independent. In this paper, we show how the Hodgkin-Huxley formalism can be extended to describe state-dependent inactivation adequately without introducing a more complex, multi-state model. This approach provides an accurate macroscopic model of cumulative inactivation of the Kv3 channel.

There is evidence that inactivation of the fast sodium channel is also state-dependent (Armstrong and Bezanilla, 1977; Bean, 1981; Aldrich et al., 1983). We use the approach developed for the Kv3 channel to provide a state-dependent description of sodium inactivation that accounts for the peculiar form of the inactivation rate constant in the Hodgkin-Huxley model and fits data on delayed inactivation (Bean, 1981).

The two currents and the general approach presented illustrate how the results of single-channel studies can be incorporated into a macroscopic model with minimal disruption of the formalism so commonly and successfully used. Once the macroscopic description has been developed, the effects of state-dependent inactivation can be studied in model neural systems. When our macroscopic model of the Kv3 current is added to a model neuron, cumulative inactivation produces an interesting short-term memory phenomenon and a firing delay similar to that produced by the current I_D in hippocampal neurons (Storm, 1988).

RESULTS

Cumulative inactivation

Fig. 1 *a* shows the macroscopic current conducted by a large number of Kv3 channels (Swanson et al., 1990) in a membrane patch in response to sustained depolarization. The current slowly inactivates (time constant 700 ms) during the course of the depolarization. From this figure alone, it might appear that inactivation of the Kv3 current could be modeled using a standard Hodgkin-

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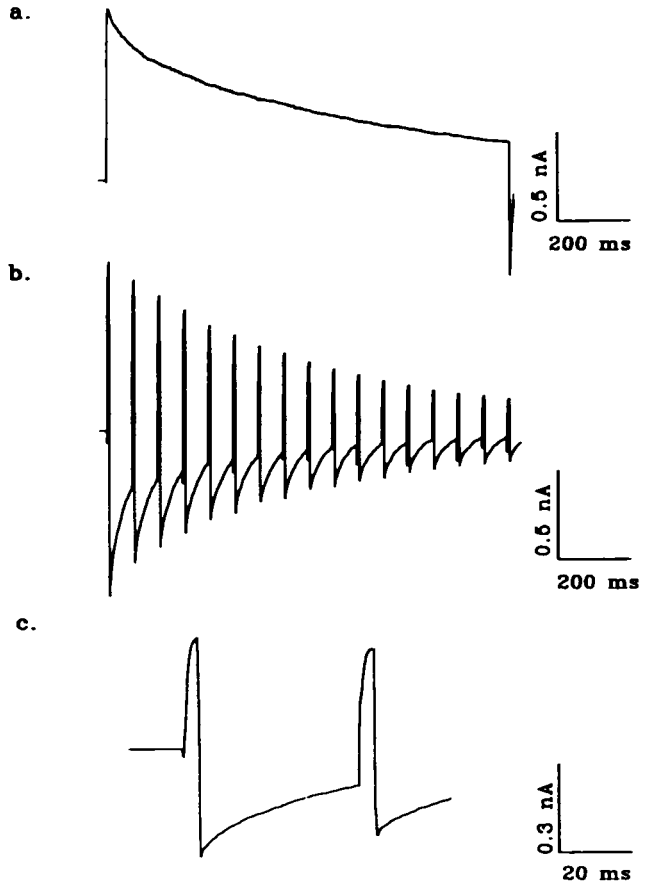


FIGURE 1 (*a*) Potassium current carried by a large number of Kv3 channels. Recorded using the detached patch-clamp configuration from a *Xenopus* oocyte that was previously injected with cRNA coding for the Kv3 channel protein (see accompanying manuscript for experimental details). The current is evoked by a depolarizing pulse from −100 mV to +40 mV for 900 ms. Solutions are as follows. External side (in mM): 30 KCl, 70 NaCl, 2 CaCl, 10 HEPES, pH 7.5; cytoplasmic side (in mM): 100 KCl, 1 EGTA, 10 HEPES, pH 7.5. (*b*) Current evoked by a series of 17 5 ms duration pulses to +40 mV, separated by inter-pulse intervals of 50 ms at −80 mV. Note the decrease in current from one pulse to the next. The current was recorded from the same patch as in *a*. (*c*) Expansion of time scale from *b* to demonstrate that no inactivation is observed within each short pulse.

Huxley description and that, because it is so slow, its biological relevance for excitable membranes is questionable. Fig. 1, *b* and *c* show that both of these assumptions are wrong. Inactivation also occurs in response to a series of short pulses of depolarization similar to a sequence of action potentials. Examination of the height of the peaks in Fig. 1, *b* and *c* also reveals that the amount of inactivation increases between the pulses even though the membrane is hyperpolarized to a level at which recovery from inactivation would normally occur. This shows that the inactivation does not depend solely on voltage and, thus, that it cannot be described using the standard Hodgkin-Huxley formalism.

In Marom and Levitan (companion manuscript), a microscopic model of the Kv3 channel was developed. Scheme 1 shows the general form of this model.

$$C_0 \underset{\beta}{\overset{4\alpha}{\rightleftharpoons}} C_1 \underset{2\beta}{\overset{3\alpha}{\rightleftharpoons}} C_2 \underset{3\beta}{\overset{2\alpha}{\rightleftharpoons}} C_3 \underset{4\beta}{\overset{\alpha}{\rightleftharpoons}} O^*, \quad O^* \rightleftharpoons I \ (k_{io}, k_{oi})$$

(Scheme 1)

The channel opens by passing through a sequence of four closed states, C_0–C_3, ultimately reaching the open state O*. In the microscopic model, O* is actually two states, but we can lump them together for purposes of the macroscopic description. Transitions between the sequence of states in the activation pathway are voltage-dependent and can be described in the usual way by an activation variable n satisfying the differential equation

$$dn/dt = \alpha_n(1 - n) - \beta_n n. \quad (1)$$

In the absence of inactivation, the number of channels in the open state O* is proportional to n^4. The voltage-dependent rate functions α_n and β_n are given by the fits obtained in Marom and Levitan (companion manuscript).

To model cumulative inactivation, we include an inactivation variable h and express the macroscopic current as $I = g_K n^4 h(V - E_K)$. Here, g_K is the maximal conductance, and E_K the potassium equilibrium potential. Normally, h would be described by an equation identical in form to Eq. 1 for n, with voltage-dependent rate constants α_h and β_h. However, this is not adequate to describe cumulative inactivation because it is a state-dependent rather than voltage-dependent process. To model cumulative inactivation, we retain the standard form for the current, $I = g_K n^4 h(V - E_K)$ but modify the form of the equation describing the inactivation variable h (see below).

In the microscopic model of Scheme 1, the channel inactivates through a voltage-independent transition from the open state O* to the inactivated state I. To model this, we note that the fraction of channels in the open state O* is n^4h in the macroscopic description, whereas the fraction in the inactivated state is $(1 - h)$. According to Scheme 1, the rate of inactivation should be equal to the fraction of open channels, n^4h, times the inactivation rate constant k_{oi}, and the rate of recovery from inactivation should be the fraction of inactivated channels $(1 - h)$ times the rate constant k_{io}. Therefore, the inactivation variable should be described by the equation

$$dh/dt = k_{io}(1 - h) - k_{oi} n^4 h, \quad (2)$$

where k_{io} and k_{oi} are *voltage-independent* rate constants, k_{io} = 1/20 s, and k_{oi} = 1/700 ms (Marom and Levitan, companion manuscript). Note that in contrast to the usual form of the inactivation equation (Hodgkin and Huxley, 1952), the membrane potential does not appear anywhere in Eq. 2. Instead, the activation variable n appears, reflecting the state dependence of the cumulative inactivation process and making this a "coupled" model (Hoyt, 1963, 1968).

The macroscopic description we have derived is not mathematically identical to the microscopic model of Scheme 1. However, it captures the essential features of the microscopic

model and provides an accurate description of the Kv3 current. Fig. 2 compares the predictions of the macroscopic model with experimental recordings of Kv3 channels. The amount of current during a train of depolarizing pulses is plotted for various interpulse intervals. There is good agreement between the data and the model, although the model predicts less inactivation for the first few pulses than is found in the data. This may be because of an additional, faster inactivation process not considered in either the microscopic or the macroscopic models. We have also compared the model with other results from Marom and Levitan (companion manuscript), such as data taken by pulsing with different interpulse holding potentials and for single, long pulses and have found similar agreement.

Voltage-independent inactivation of the sodium channel

The sodium channel modeled so successfully by Hodgkin and Huxley (1952) does not actually function in a manner consistent with their model (Armstrong and Bezanilla, 1977; Bean, 1981; Aldrich et al., 1983). In particular, measurements of gating currents (Armstrong and Bezanilla, 1977) and of single channels (Aldrich et al., 1983) indicate that the inactivation transition is largely voltage-independent. Following the example of cumulative inactivation in Kv3, we can modify the standard description of the sodium channel to account for voltage-independent inactivation.

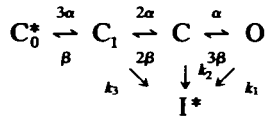
The standard macroscopic description of the fast sodium conductance uses an activation variable m and an inactivation variable h to express the sodium current as $I = g_{Na}m^3h(V - E_{Na})$. The activation variable satisfies the differential equation

$$dm/dt = \alpha_m(1 - m) - \beta_m m \quad (3)$$

and, in the conventional model,

$$dh/dt = \alpha_h(1 - h) - \beta_h h. \quad (4)$$

In contrast, microscopic descriptions of sodium channel inactivation use models of the form shown below (Hille, 1992; Patlak, 1991):



(Scheme 2)

In this scheme, states marked with an asterisk are multiple states in the microscopic model that can be lumped together for our purposes. The data suggest that the rate constants k_1, k_2, and k_3, describing transitions to the inactivated state I*, are voltage-independent. As shown in the scheme, the inactivated state can be reached from the fully activated open state O as well as from the closed, but partially activated, states C_1 and C_2. The chain of states leading to activation is similar to the activation pathway in the Hodgkin-Huxley model, and we will not modify their description of the activation process. In the macroscopic description, the fraction of channels in the open state O is m^3h, the fraction in state C_2 is $3m^2(1 - m)h$ and in the state C_1, $3m(1 - m)^2h$. Thus, the voltage-independent transitions from these three states to the inactivated state have rates k_1m^3h, $3k_2m^2(1 - m)h$ and $3k_3m(1 - m)^2h$. According to our general approach, the inactivation process should thus be described by the equation

$$dh/dt = \alpha_h(1 - h) - [k_1m^3 + 3k_2m^2(1 - m) + 3k_3m(1 - m)^2]h \quad (5)$$

instead of the usual voltage-dependent equation for h, Eq. 4. Note that the term within the square brackets in Eq. 5 that replaces β_h is completely independent of voltage, but depends on the occupancy of the last three states in the activation pathway. We have not changed the usual description of recovery from inactivation, so we retain the normal, voltage-dependent recovery rate constant $\alpha_h(V)$.

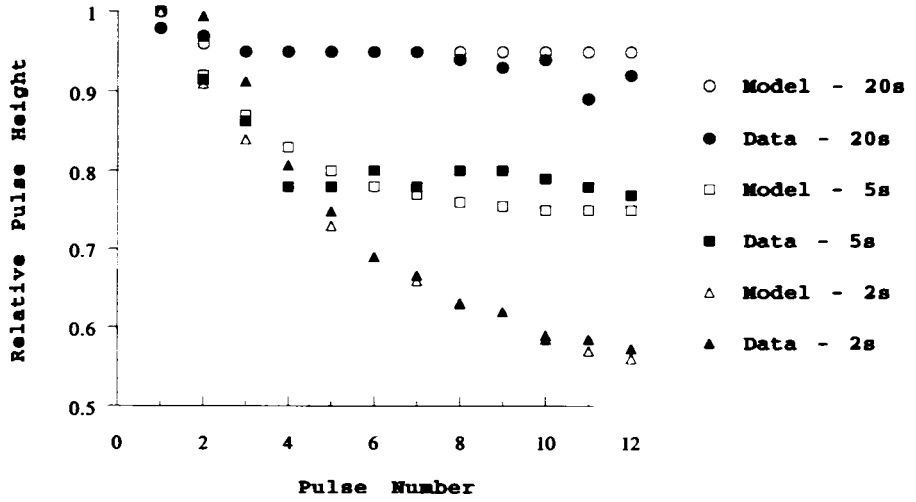


FIGURE 2 Comparison of macroscopic model of Kv3 current with data. A series of 15 ms voltage clamp pulses from a holding potential of −90 mV to a pulse potential of +40 mV were applied with interpulse intervals ranging from 2 to 20 s. The relative amplitude of the resulting current pulses is plotted. Data were obtained as in Fig. 1. Model results were obtained by numerically integrating the equations discussed in the text.

As in the case of the Kv3 current, the state-dependent macroscopic model we have introduced is not equivalent to the microscopic model but, rather, it captures the essential features of the microscopic description with regard to the macroscopic current.

Can the new form of Eq. 5 fit data on the sodium channel? We begin by comparing steady-state inactivation in the two approaches. At steady state, $m = m_\infty(V)$. If the two approaches are to agree, we must have

$$\beta_h = k_1 m_\infty^3 + 3k_2 m_\infty^2(1 - m_\infty) + 3k_3 m_\infty(1 - m_\infty)^2 \qquad (6)$$

at all voltages V. This is equivalent to fitting the function $\beta_h(V)$ to a cubic polynomial in the function $m_\infty(V)$ with three free-fitting parameters k_1, k_2, and k_3. Is such a fit possible? At first sight, this would appear unlikely. The function $m_\infty(V)$ is of a sigmoidal form and only varies strongly when V is in the range $-60 < V < -10$ (see Fig. 3). Rate functions are usually exponentials, or at least strongly varying functions of V over the entire voltage range where they are appreciably different from zero. It would be impossible to fit α_m, β_m, α_n, β_n, or α_h to a cubic polynomial of m_∞ because they have the wrong shape. However, the Hodgkin-Huxley fit to β_h is, remarkably, a sigmoidal function that varies over almost exactly the same range as m_∞. This is shown in Fig. 3. An extraordinarily good fit (correlation coefficient 0.99991) can be obtained with $k_1 = 1/1.0$ ms, $k_2 = 1/2.3$ ms, and $k_3 = 1/4.0$ ms. This fit, shown in Fig. 3, is virtually indistinguishable from the original rate function β_h.

Although the modified model agrees almost exactly with the Hodgkin-Huxley model for the steady-state inactivation, the kinetic form of the inactivation process is different. Fig. 4 shows the time course of inactivation during a voltage-clamp pulse from a hyperpolarized to a depolarized potential. The Hodgkin-Huxley model predicts that, under such circumstances, inactivation will begin immediately and h will fall exponentially from its initial value of one. As a result, the time derivative of the inactivation, dh/dt, is predicted to be nonzero at time $t = 0$ when the depolarizing pulse begins. Precise measurements have revealed a short delay before the sodium inactivation process begins following depolarization (Bean, 1981; Goldman and Schauf, 1972; Bezanilla and Armstrong, 1977). Although nonzero, this delay is shorter than the delay to maximum activation of the sodium current (Bean, 1981). Fig. 4 shows that the coupled model we have derived agrees with the double-pulse data. The inactivation process is delayed in the model because it cannot proceed until some activation has occurred. Initially at $t = 0$, $dh/dt = 0$ and dh/dt reaches a maximum before the maximum of the sodium current just as it does in the double-pulse experiments (Bean, 1981).

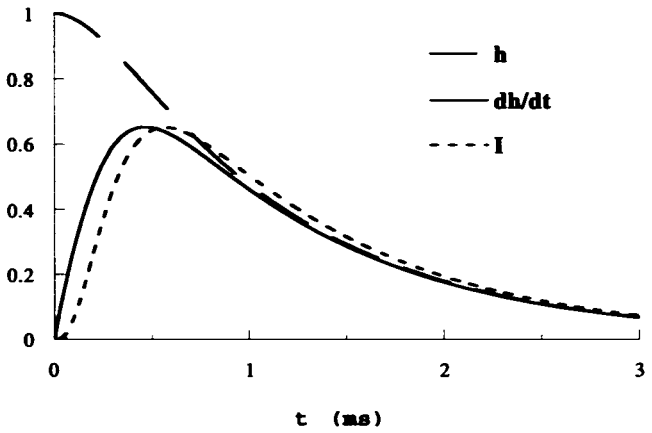


FIGURE 4 Inactivation delay in the state-dependent model. The inactivation variable h and its rate of change dh/dt are plotted against time after a rapid depolarization (at $t = 0$) to +10 mV from a holding potential of −100 mV. The short-dashed curve is the total sodium current normalized so that its height matches that of dh/dt. For h, the y axis is in dimensionless units; for dh/dt, the y axis is in units of 1/ms, for I, it is in arbitrary units of current. This figure demonstrates that the inactivation process is delayed, but the delay is less than that of the full current. Compare with Fig. 11 of Bean (1981).

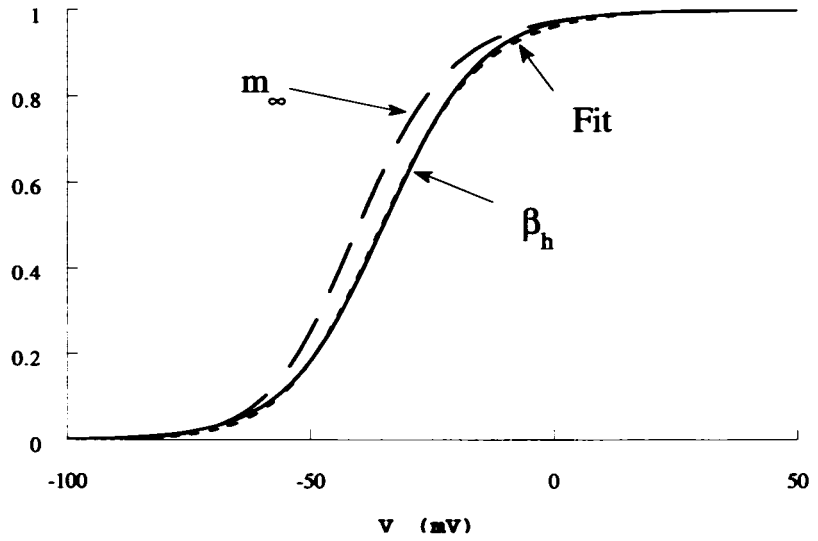


FIGURE 3 Comparison of two expressions for the inactivation rate of the sodium current. The line with long dashes is m_∞, the steady-state activation of the sodium current, and β_h is the voltage-dependent inactivation rate of the Hodgkin-Huxley model (1952). The curve with short dashes marked Fit is the best fit of the cubic polynomial in m_∞ discussed in the text to the function β_h. For the curve m_∞, the y axis is in dimensionless units, whereas for β_h and "Fit" the y axis is in units of 1/ms.

Implication of cumulative inactivation for temporal integration

The modified description of the sodium current that we have developed does not differ from the normal description sufficiently to affect neuronal behavior dramatically. However, the situation is quite different for the Kv3 current. We can use the macroscopic description we have developed to study how the unusual properties of the Kv3 channel affect the behavior of a model neuron. The effects we describe arise from the cumulative nature and slow recovery of the Kv3 inactivation; the voltage independence of the inactivation process is not essential. We use a single-compartment model with fast sodium, delayed rectifier potassium, and chloride leakage currents. We add to this model the Kv3 current as described above. The resulting behavior is shown in Fig. 5.

The Kv3 current deactivates quite slowly and as a result it significantly reduces the firing rate of the model neuron.

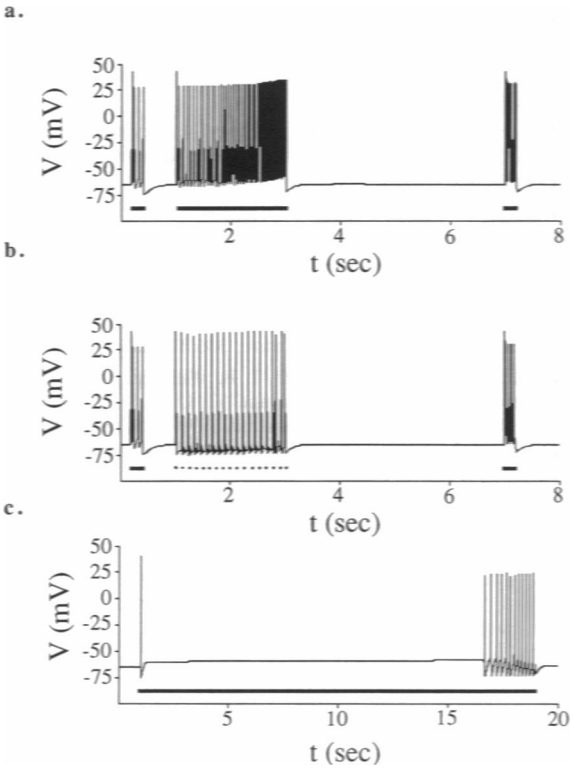


FIGURE 5 (*a*) Kv3-induced "memory" effect in a model neuron. The model has 20 mS/cm^2 maximal sodium conductance, 3 mS/cm^2 maximal delayed rectifier potassium conductance, and 0.5 mS/cm^2 maximal Kv3 conductance. A 2 nA test pulse of injected current lasting for 200 ms caused the model neuron to fire slowly (*first bar*). This was followed by 2 s of sustained 2 nA current injection (*longer bar*). After a 4-s pause, a test pulse (*third bar*) identical to the first pulse produced a much higher firing rate because of residual inactivation of the Kv3 current. (*b*) Effect similar to that in *a* produced by intermittent current injection. Intermittent injection, denoted by the dashed bar, consisted of 5-ms pulses of 2 nA every 50 ms for 2 s. Same model and parameters as in *a*. (*c*) Delay to sustained firing. An 18-s injection of 1.5 nA produced an initial spike but no sustained firing until the Kv3 current partially inactivated. Same model and parameters as in *a*, except that the maximal conductance of Kv3 is 3 mS/cm^2. Compare with Fig. 1 of Storm (1988).

During the first current pulse shown in Fig. 5 *a*, the model neuron fired at around 15 Hz, which is much slower than its firing rate in the absence of Kv3 (as high as 100 Hz for this amount of injected current). After the initial short test pulse in Fig. 5 *a*, we injected a longer 2 s current pulse. The firing rate increased steadily during this pulse as cumulative inactivation decreased the effect of the Kv3 current. By the end of the 2-s pulse, the firing rate was above 50 Hz. Recovery from inactivation of the Kv3 channel is extremely slow (recall k_{io} = 1/20 s). As a result, the Kv3 current will remain in the inactivated state for many seconds after sustained firing. The last current pulse in Fig. 5 *a* is another test pulse identical to the first one shown in the figure. This pulse occurred 4 s after the 2-s period of sustained firing ended, but the effect of that firing was still apparent. The Kv3 current was still partially inactivated, and in response to the test pulse, the model neuron fired at 40 Hz as opposed to the 15 Hz rate seen for the first test pulse.

Because inactivation of the Kv3 current is a cumulative process, the long, steady depolarization used in Fig. 5 *a* is not needed to induce the increased response to a test pulse. In Fig. 5 *b*, a series of short depolarizing pulses was used instead to produce a similar effect as in Fig. 5 *a*. Indeed, any prolonged activity, whether caused by steady or fluctuating stimulation, will inactivate the Kv3 current and induce the long-lasting increase in excitability seen in Fig. 5, *a* and *b*.

In Fig. 5 *c*, a model neuron with a larger maximal Kv3 conductance was depolarized just below its threshold for repetitive firing. After an initial spike, no further spiking occurred for several seconds. However, during this time the Kv3 current partially inactivated and, after a sufficient time had passed, repetitive spiking began. This is a particularly interesting phenomenon because a very similar effect has been observed in hippocampal neurons (Storm, 1988) and has been attributed to the current I_D.

DISCUSSION

Microscopic models of membrane channels attempt to describe macroscopic, single-channel, and gating currents to provide a mechanistic description of how a channel operates. Modelers dealing with the effects of different membrane currents on neuronal and network behavior are primarily interested in an accurate description of macroscopic currents. Nevertheless, this might require that information from microscopic studies be incorporated into the macroscopic description. Through the two different examples studied, we have demonstrated a method for doing this. In the case of cumulative inactivation, this approach is essential to achieve a macroscopic description that is even qualitatively correct. In the case of sodium current inactivation, the differences between the description we have provided and the standard approach are fairly subtle. Nevertheless, it is interesting to see that when reinterpreted in the state-dependent model, the unusual sigmoidal shape of the inactivation rate function β_h

of the original Hodgkin-Huxley fits (1952) provides evidence for voltage-independent inactivation.

The general approach we have presented can be applied readily to other examples. Once the sequence of states and allowed transitions are identified in a microscopic model, the macroscopic description of the fraction of states occupied can be used to derive rate equations for the inactivation and activation variables. This extends the standard neuron modeling formalism from a purely voltage-dependent description to include state-dependent processes.

When included in a simple model of a spiking neuron, the macroscopic Kv3 current produces the interesting behavior seen in Fig. 5. Because of cumulative inactivation and extremely slow recovery from inactivation, the Kv3 current can provide a neuron with a short-term memory of past activity. A neuron with even a relatively low density of Kv3 channels that has experienced sustained firing within the past 10–20s, will respond much more vigorously to a new input than one that has been silent. A somewhat higher level of Kv3 current produces effects very similar to those attributed to the current I_D (which appears to have properties similar to those of the Kv3 current) in hippocampal neurons (Storm, 1988). Using the macroscopic model of the Kv3 current, this form of temporal integration and the associated neuronal "short-term memory" can be studied in network models as well.

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