A simple modification of the Hodgkin and Huxley equations explains type 3 excitability in squid giant axons

John R. Clay¹,⁴*, David Paydarfar² and Daniel B. Forger³

¹National Institute of Neurological Disorders and Stroke, National Institutes of Health, Twinbrook Building, Rm TN-41, 5625 Fishers Lane, Bethesda, MD 20892, USA
²Department of Neurology and Physiology, University of Massachusetts Medical School, Worcester, MA 01655, USA
³Mathematical Biology Research Group, Department of Mathematics, Center for Computational Medicine and Biology, University of Michigan, Ann Arbor, MI 48109, USA
⁴Marine Biological Laboratory, Woods Hole, MA 02543, USA

The Hodgkin and Huxley (HH) model predicts sustained repetitive firing of nerve action potentials for a suprathreshold depolarizing current pulse for as long as the pulse is applied (type 2 excitability). Squid giant axons, the preparation for which the model was intended, fire only once at the beginning of the pulse (type 3 behaviour). This discrepancy between the theory and experiments can be removed by modifying a single parameter in the HH equations for the K⁺ current as determined from the analysis in this paper. K⁺ currents in general have been described by $I_K = g_K(V - E_K)$, where $g_K$ is the membrane’s K⁺ current conductance and $E_K$ is the K⁺ Nernst potential. However, $I_K$ has a nonlinear dependence on $(V - E_K)$ well described by the Goldman–Hodgkin–Katz equation that determines the voltage dependence of $g_K$. This experimental finding is the basis for the modification in the HH equations describing type 3 behaviour. Our analysis may have broad significance given the use of $I_K = g_K(V - E_K)$ to describe K⁺ currents in a wide variety of biological preparations.

Keywords: nerve; squid axons; ion channel gating; neuronal excitability

1. INTRODUCTION

In 1948, Alan Hodgkin described three classes or types of axonal excitability in experiments on crustacean nerves using constant current stimuli (Hodgkin 1948). Axons having type 1 excitability fired repetitively with a frequency that depended markedly on stimulus intensity. Type 2 axons also fired repetitively once a threshold had been crossed but with a frequency that depended relatively little on stimulus intensity. Type 3 axons fired at most once or twice, or not at all regardless of stimulus intensity or duration. Squid giant axons exhibit type 3 excitability (Clay 1998), which is surprising since the Hodgkin & Huxley (HH 1952d) equations of this preparation describe type 2 behaviour. The model and the underlying voltage-clamp measurements of ionic current in squid giant axons are well known (HH 1952a–d). In particular, their description of nerve excitability contains separate pathways for sodium and potassium ions, a result that foreshadowed the discovery of ion-specific channels (Hille 2001), and they performed a detailed kinetic analysis of Na⁺ and K⁺ currents, which continues to serve as a paradigm for the manner in which voltage-clamp experiments are carried out. Finally, HH (1952d) used their equations to simulate membrane electrical behaviour, in particular the action potential response to brief duration current pulses. They cautioned against applications of their equations for long time scales, although Huxley (1959) and others have used the model for that purpose. The model predicts steady repetitive firing, type 2 (Rinzel 1978), whereas the squid giant axon fires only once at the beginning of the pulse and is then quiescent for the remainder of the pulse regardless of the pulse amplitude or duration, i.e. type 3 excitability.

Type 3 excitability in squid axons was analysed in an earlier work from this laboratory (Clay 1998), although the term type 3 was not used in that report. In that work, the discrepancies between the HH (1952d) model of Na⁺ channel gating and the experimental measurements of this component following their work (reviewed by Patlak 1991) were considered to be important for models of squid axon excitability, in particular the type 3 result. We have since come to the conclusion that $\dot{I_{Na}}$ may not be a significant factor for this result. The HH (1952d) $\dot{I_{Na}}$ model is sufficient (see §4). Given that their model is simpler than modern models of Na⁺ channel gating, we have reverted in this report to the original

*Author for correspondence (jrclay@ninds.nih.gov).
HH (1952d) equations and focused on modifications of the $I_K$ component which are critical for type 3 excitability. These results were also a part of the earlier analysis (Clay 1998), but were obscured by the emphasis on $I_{Na}$.

Here, we show that the discrepancy between the HH (1952d) model and experiments concerning the type 3 result can be resolved by changing a single parameter in their equations for $K^+$ current gating based on the $K^+$ current recordings in this paper. The genesis of the modification is surprising. Consider the form for $K^+$ current given by HH (1952b), $I_K = g_K(V, t)(V - E_K)$, where $g_K$ is the time- and voltage-dependent $K^+$ conductance, $V$ is the membrane potential and $E_K$ the Nernst potential for potassium ions, which for most biological cells is in the $-70$ to $-90$ mV range. This equation cannot be correct given the potassium ion gradient across the membrane. The potassium ion concentration inside the biological cells, $[K^+]_i$, is significantly greater than the external concentration, $[K^+]_o$. The $K^+$ current for potentials positive to $E_K$ is carried principally by intracellular $K^+$, whereas the current for potentials below $E_K$ is carried principally by extracellular $K^+$. The slope conductance for $V < E_K$ is not the same as that for $V > E_K$, given that $[K^+]_i > [K^+]_o$. Instead, the current–voltage relation has a nonlinear dependence on $(V - E_K)$, which is well described by the Goldman–Hodgkin–Katz (GHK) equation (Goldman 1943; Hodgkin & Katz 1949; Frankenhauser 1962; Binstock & Goldman 1971; Clay 1991). This nonlinearity, in turn, influences measurements of the voltage dependence of $g_K$. In this paper, we fit the GHK equation to experimental recordings of $I_K$, which leads to a more accurate determination of one of the parameters in the HH (1952d) model of this component, a modification that makes the model more closely resemble experimental results concerning squid axon excitability.

2. METHODS

Experiments on squid giant axons were carried out at the Marine Biological Laboratory in Woods Hole, Massachusetts, using axial wire current and voltage-clamp methods previously described (Clay & Shlesinger 1983). The external solution was filtered seawater. The temperature of the experiments was in the 6–8°C range. In any given experiment, it was maintained constant to within 0.1°C by a Peltier device located within the experimental chamber. For recording potassium ion current, $I_K$, the sodium ion current, $I_{Na}$, was blocked by the addition of tetrodotoxin to the external medium. Simulations were carried out with the original HH (1952d) model and with the revised version of the model, both given in appendix A.

3. RESULTS

3.1. Type 3 excitability

The response of squid giant axons to sustained suprathreshold current pulses is a single spike, or action potential, followed by quiescence for the remainder of the pulse regardless of pulse amplitude or duration—type 3 behaviour (figure 1). The resting potential of this preparation was $-61$ mV, the maximum overshoot of the spike was $31$ mV, the undershoot was $-68$ mV and the steady quiescent level throughout the duration of the pulse was $-58$ mV. Similar results were observed in all experiments ($n = 20$ axons) for pulse durations up to 2 s and for pulse amplitudes up to $50$ mA cm$^{-2}$. These results are consistent with the experimental observations of type 3 excitability in previous reports (Clay 1998, 2005).

3.2. $I_K$ recordings

Representative recordings of $I_K$ are shown for three different voltage steps ($V_{step} = -25, -5$ and $5$ mV from a holding potential of $-75$ mV (figure 2). A rest interval of at least $3$ s was used between each step. The initial times of the records are offset from one another. The currents at the end of each step, $I_K$, are indicated by the respective horizontal bars. $T = 8.8$°C.

Figure 1. Response of a squid giant axon, type 3, to a sustained current pulse $15$ μA cm$^{-2}$ in amplitude. $T = 8$°C.

Figure 2. Three different $I_K$ recordings from a squid giant axon preparation (Clay & Shlesinger 1983) taken from a family of recordings in which the holding potential was $-75$ mV, and the step potential ($V_{step}$) ranged from $-55$ to $+35$ mV in $10$ mV increments with a $3$ s rest interval between each step. The sodium ion current, $I_{Na}$, was blocked by tetrodotoxin (1 μM; Sigma Chemical Co., St Louis, MO). The initial times of each step are offset from one another. The currents at the end of each step, $I_K$, are indicated by the respective horizontal bars. $T = 8.8$°C.
membrane potential, \( E_K \) is the K\(^+\) Nernst potential and \( d n(V, t) / dt = - (\alpha_n + \beta_n) n(V, t) + \alpha_o , \) where \( \alpha_o \) and \( \beta_o \) are voltage dependent (HH 1952\(^d\); see appendix A). The solution to this equation for a voltage step to a depolarized level, such as \(-25 \text{ mV},\) is an exponential function of time. Raising that solution to the fourth power yields a sigmoidal time dependence similar to that of the experimental recordings. The current at the end of each record, \( I_o \) is indicated by a horizontal bar (figure 2). In the HH (1952\(^d\)) model, \( I_o = g_K n^2(V_{\text{step}})(E_{\text{step}} - E_K) , \) where \( n^2(V_{\text{step}}) = (\alpha_o (V_{\text{step}})/(\alpha_o + \beta_o ))^2 , \) a parameter that varies between zero at relatively negative potentials, such as the holding level, and unity at strongly depolarized potentials. This function is referred to as the \( I_o \) activation curve or \( g_K - V \) curve. HH (1952\(^b\)) obtained this relation from normalization of their \( I_o \) results with \( (V_{\text{step}} - E_K) , \) since they assumed \( I_o \) was directly proportional to \( (V - E_K) . \) However, \( I_o \) is, instead, proportional to the GHK dependence on \( (V - E_K) \) (Goldman 1943; Hodgkin & Katz 1949), as shown here (figure 3) and elsewhere (Clay 1991). These results (figure 3) were obtained with a 30 ms prepulse to \(-90 \text{ mV} \) followed by steps to the potentials indicated on the abscissa. The currents immediately following that second step are plotted. The data have a nonlinear dependence on \( (V - E_K) , \) which is well described by the GHK equation (figure 3). We have concluded from these results that \( I_o \) is given by

\[
I_o = n^4(V, t) P_K F[K^+](qV/kT)(\exp(q(V - E_K)/kT - 1)) \tag{3.1}
\]

where \( P_K \) is the membrane permeability of K ions; \( F \) is the Faraday constant; \( q \) is the unit electronic charge; \( k \) is the Boltzmann constant; and \( T \) is the absolute temperature \( (kT/q = 24 \text{ mV at } T = 7^\circ \text{C}) . \) Equation (3.1), the part after \( n^4(V, t) , \) was derived from macroscopic diffusion theory (Goldman 1943). At the microscopic level, potassium ions move through K\(^+\) channels via single file diffusion along a row of three sites (Hodgkin & Keynes 1955; Zhou et al. 2001). At this level, an expression comparable to equation (3.1) can be derived (Clay 1991). Specifically,

\[
I_o = n^4(V, t) \delta Q_n \exp(-2d_1 + 2d_2 V/kT)(\exp(q(V - E_K)/kT - 1)) \tag{3.2}
\]

where \( \delta \) is a constant related to the frequency of collisions of K ions with the channel; \( N_K \) is the K\(^+\) channel density; \( d_1 = 0.07 ; \) and \( d_2 = 0.18 . \) The voltage dependence of equation (3.2) is virtually indistinguishable from that of equation (3.1) for \(-150 < V < +150 \text{ mV} \) (Clay 1991), and so we use

\[
I_o = n^4(V, t) \delta Q_n GHK((V - E_K)) \tag{3.3}
\]

or \( I_o = n^4(V, t) \delta Q_n GHK((V - E_K)) \) for brevity. At the single channel level in the HH model \( I_o = n^4(V, t) \gamma K \times N_K(V - E_K) , \) where \( \gamma K \) is the K\(^+\) channel conductance.

Figure 3. Nonlinear dependence of \( I_o \) on \((V - E_K)\). In this preparation, the membrane potential was stepped from a holding level of \(-90 \text{ to } -30 \text{ mV} \) for 30 ms followed by a 10 ms step to the potentials indicated on the abscissa. The currents immediately following the second step are indicated by the circles. The curve is a fit to these results by the GHK equation (equation (3.1)), \( I_o = \phi(qV/kT)(\exp(q(V - E_K)/kT - 1))/\exp(qV/kT - 1) , \) where \( \phi \) is a constant, \( kT/q = 24 \text{ mV} \) and \( E_K = -72 \text{ mV} \). The only adjustable parameter of the fit is \( \phi \). Results are taken from Clay (1989).

Figure 4. Voltage dependence of \( g_K \) activation. The circles correspond to the experiment illustrated in figure 1. The \( I_o \) results from those records were normalized by GHK[(\( V - E_K)\)]. The values of that procedure reached saturation at \(+5 \text{ mV} \). The points at 5, 15, 25 and 35 mV were averaged, and that average value was used to normalize the results a second time so that this relation for \( V \geq 5 \text{ mV} \) corresponds to unity. The curve labelled ‘HH \( n_z^2(V) \)’ represents \( n_z^2(V) \) in the HH (1952\(^d\)) model, where \( n_z(V) = \alpha_o / (\alpha_o + \beta_o) . \) The curve labelled ‘\( n_z^2(V) \) revised’ is the prediction of the model using the modified \( \beta_o \) described in the text \((V_o = 19.7 \text{ mV} \) as opposed to \( 80 \text{ mV} \) in the HH model).

The current at the end of voltage steps, such as those in figure 2, is given by \( I_o = n_z^2(V) \delta Q_n GHK[(V - E_K)] \). The first step in obtaining \( n_z^2(V) \) in the revised model is to normalize \( I_o \) by GHK[(\( V - E_K)\)]. For example, \( GHK[(V - E_K)] = 9.8 \) is a dimensionless quantity, for \( V = V_{\text{step}} = -25 \text{ mV} \) and \( I_o = 0.8 \text{ mA cm}^{-2} \) (figure 1), giving a ratio of 0.082 mA cm\(^{-2} \). Similar results for all the voltage steps (figure 4) describe a sigmoidal relation which saturates for \( V_{\text{step}} > 0 \text{ mV} \) with \( 0.151 \text{ mA cm}^{-2} \) as a second normalization factor to give the \( g_K - V \) curve. The HH model does not adequately describe these results (figure 4). The challenge we faced was to alter their model to fit the \( g_K - V \) curve.

\[ J. R. Soc. Interface (2008) \]
The predictions of their model using the modified curves in (V

with I
dotted lines are the data.

J. R. Soc. Interface

1424 Type 3 excitability in squid giant axons J. R. Clay et al.

Figure 5. Description of \( I_K \) kinetics. The data are the same as in figure 2 for (a) \( V_{step} = -5 \) and (b) \(-25 \text{ mV}\). The theoretical curves correspond to \( I = (n_a(V_{step}) - n_a(V_h))\exp(-(\alpha_n(V_{step}) + \beta_n(V_{step}))t) \) \((\text{figure 5})\), where \( V_h = -75 \text{ mV} \), \( n_a(V_{step}) = \alpha_n(V_{step})/(\alpha_n(V_{step}) + \beta_n(V_{step})) \), \( n_a(V_h) = \alpha_n(V_h)/(\alpha_n(V_h) + \beta_n(V_h)) \) and \( I \). \( n_a(V_{step}) = I_0 \) (figure 1). The red curves in (a) and (b) correspond to the respective predictions of the HH (1952d) model. The blue curves in both (a) and (b) are the predictions of their model using the modified \( \beta_n \). The black dotted lines are the data.

curve without significantly modifying \( I_K \) activation kinetics, which are well described by their model. Those results are determined primarily by \( \alpha_n \), for which they used \( \alpha_n = -0.01(V + 50)/\exp(-0.1(V + 50)) - 1 \text{ ms}^{-1} \). Their expression for \( \beta_n \) is \( 0.125 \exp(-(V + 60)/V_h) \text{ ms}^{-1} \), where \( V_h = 80 \text{ mV} \). The \( \beta_n \) parameter is significant in determining the \( g_K \)-V curve and deactivation, or 'tail' current kinetics. HH did not report the latter. The voltage dependence of those results is not consistent with their choice of \( \beta_n \) (Clay 1984). Both sets of results are well described following the modification of \( V_0 \). We used a least-squares procedure to obtain \( V_0 \) by fitting the HH (1952d) model of \( n_a(V) \) to the results in figure 4 with \( V_h \) as the only adjustable parameter. The result was \( V_h = 19.7 \text{ mV} \) (figure 4), which steepened the \( g_K \)-V curve and shifted its midpoint from \(-14.2 \text{ mV} \) in the HH model to \(-36 \text{ mV} \) in the revised version. The modified \( V_0 \) also provides a good fit to activation kinetics (figure 5). Those results are determined primarily by \( \alpha_n \), as noted above. However, the starting value for \( n \), \( n_c \), is affected by \( \beta_n \), since \( n_c = \alpha_n(V_h)/(\alpha_n(V_h) + \beta_n(V_h)) \), with \( V_h = -75 \text{ mV} \) and \( \beta_n \) is greater than \( \alpha_n \) at this potential. The modified \( \beta_n \) alters the delayed onset of activation in the model following a voltage step so that the predictions of the model closely match the experimental records (figure 5).

The modified \( \beta_n \) partially explains the results of Cole & Moore (1960) concerning the influence of the holding, or starting, potential on the delay in activation of \( I_K \). They showed that even modest hyperpolarizations such as \(-75 \text{ mV} \) produced a delay in the onset of \( I_K \), which was not described by HH (1952d). This effect is illustrated in figure 5. The experimental results (the black curves) rise in a manner similar to the HH predictions (the red curves) but only following a delay relative to HH. As noted above, this discrepancy for \( V_h = -75 \text{ mV} \) is removed with the modified \( \beta_n \) in the model (the blue curves). The revised model describes the delayed onset with starting potentials as negative as \(-100 \text{ mV} \) (results not shown). The Cole–Moore delay increases essentially without limit down to \(-250 \text{ mV} \) (Cole & Moore 1960; Clay & Shlesinger 1982). The discrepancy between the delay in activation in the revised model and the experimental recordings of \( I_K \) begins to occur for starting potentials below \(-100 \text{ mV} \), which is outside the physiological range, in particular the range of potentials spanned by an action potential. Stated differently, the modification in \( \beta_n \) as determined from GHK analysis is sufficient to modify the HH model from type 2 to type 3 behaviour. Serendipitously, this modification also describes the delay in \( I_K \) activation kinetics over the physiological range of membrane potentials.

3.3. The revised model has type 3 dynamics

The effect of changing \( \beta_n \), and only \( \beta_n \) (i.e. steepening the \( I_K \) activation curve), in the HH (1952d) model is illustrated in figure 6. A long-lasting current pulse elicits a repetitive train of action potentials from the original model for as long as the pulse is applied over a broad range of pulse amplitudes, i.e. type 2 excitability. Only a single action potential is elicited from the revised model, i.e. type 3 excitability. In these simulations, we used \( I_K = g_K n(V, t)(V - E_K) \), for simplicity, with all parameters as in the HH (1952d) model except for the change in \( \beta_n \) (modified \( V_0 \)) rather than \( I_K = \delta g_N k n(V, t) GHK[(V - E_K)] \) also with the modified \( \beta_n \). This result appears to counter our emphasis on the use of the GHK equation. The GHK[(V - E_K)] relation differs relatively little from the straight line relation for \(-70 < V < -30 \text{ mV} \) (figure 3). Consequently, the simulations in the revised model are essentially indistinguishable from one another regardless of whether \( I_K \sim (V - E_K) \) or \( I_K \sim GHK[(V - E_K)] \) is used because the membrane potential spends very little time outside the \(-70 < V < -30 \text{ mV} \) range during an action potential. The difference between GHK and the straight line relation is even less for the \(-65 \) to \(-55 \text{ mV} \) range, which, as shown below, is central for the revision of the
HH model from type 2 to type 3. However, the relative conductances of the two models in this voltage range are determined via the normalization procedure with currents measured for $V > 0$ mV. In this voltage range, $\text{GHK}(V - E_{\text{K}})$ differs substantially from $(V - E_{\text{K}})$, which accounts for the difference in the initial rising phase of the activation curves (the foot of the curves) in the $-65$ to $-55$ mV range (figure 4)—the key factor in the type 2-type 3 analysis (see below). The GHK $((V - E_{\text{K}}))$ relation must be used for descriptions of K$^+$ current families, such as the results in figures 2 and 5, but not for simulations of excitability.

3.3.1. Ionic basis of type 3 behaviour. Insight into the mechanism of the type 3 result (as well as type 2 behaviour) can be obtained by superimposing the initial portions of the voltage waveforms in figure 6 for the HH and revised models (figure 7a). The action potential duration of the revised model is slightly less than that of the HH model owing to increased activation of $I_{\text{K}}$ during the spike (steeper activation of $I_{\text{K}}$ which, in turn, causes the membrane potential to remain in the vicinity of the foot of the spike longer in the revised model. This effect, paradoxically, results in a greater depolarization towards threshold in the revised model is nearly the same in both (figure 8c). In HH, the well-known regenerative activation of $I_{\text{Na}}$ (Hille 2001) begins to occur near the end of the simulation (figure 8c). Indeed, the membrane potential is beginning its upward rise towards the upstroke of the second action potential (figure 8a). The increased activation of $I_{\text{K}}$ in the revised model prior to threshold (produced by the modification in the $g_{\text{K}} - V$ curve) prevents further activation of $I_{\text{Na}}$, i.e. failure of additional spikes.

The above analysis demonstrates that the differences between types 2 and 3 models are subtle. Both the models have a threshold for a single spike in response to a brief duration pulse owing to the resting $I_{\text{K}}$ level which also produces a threshold for repetitive firing in the HH model for a sustained current pulse. Firing does not occur below the single spike threshold. That is, the firing frequency for sustained stimuli jumps from zero near threshold to a finite value in type 2 excitability, a frequency which is relatively insensitive to further increases in stimulus amplitude owing to the residual $I_{\text{K}}$ that remains after a spike. Type 1 excitability occurs, or can occur, in cells having a rapidly inactivating K$^+$ component, $I_{\Lambda}$, a component which is not present in squid axons (Connor et al. 1977). The addition of $I_{\Lambda}$ to the HH model causes the threshold to repetitive firing in response to sustained current to be less than in HH and to produce a firing rate that depends on current amplitude more than in HH (Connor et al. 1977).

4. DISCUSSION

Squid giant axons have firing properties that are consistent with the type 3 classification scheme of Hodgkin (1948). The type 3 crustacean axons in his
report fired once or twice, or not at all. He suggested that these results may have been attributable to rundown of the preparations. Type 3 behaviour was consistently observed in our experiments with freshly dissected axons having an action potential amplitude of 100 mV (figure 1), or higher. In the earlier model of type 3 behaviour from this laboratory (Clay 1998), the multi-state Markov chain model of Na⁺ channel gating in squid axons by Vandenberg & Bezanilla (1991b) was used. Their model describes I₉,Na voltage-clamp results not predicted by HH (1952d), in particular observations obtained with a double-voltage-step protocol suggesting that Na⁺ channel activation and inactivation are kinetically linked (Bezanilla & Armstrong 1977). These processes are independent of each other in the HH (1952d) model. Those results may not be relevant for membrane excitability. The single step protocol used by HH (1952a-c) appears to be sufficient, results that are well described by the HH (1952d) I₉,Na model (Oxford 1981; J. R. Clay 2001, unpublished simulations). We have found that type 3 behaviour occurs with either model of I₉,Na, given the change in the βₚ parameter described above. Accumulation and depletion of K ions in the extracellular space between the axolemma and the glial cells surrounding the axon, the so-called Frankenhauser & Hodgkin (1956) space, was also included in the earlier work (Clay 1998). That portion of the model is not a factor in type 3 behaviour (simulations not shown).

Several groups have recently used the GHK normalization procedure described in this study and in an earlier report (Clay 2000) to obtain K⁺ channel activation curves (DeFazio & Moenter 2002; Boland et al. 2003; Van Hoorick et al. 2003; Persson et al. 2005; Nakamura & Takahashi 2007; Johnston et al. 2008). A far larger number of reports have used normalization with \((V−E_{K})\). Many of these results have been from K⁺ channels heterologously expressed in *Xenopus* oocytes. A determination of the relationship of those results to excitability in the native cells containing the relevant K⁺ channels is not straightforward. Other reports have been from native cells in which analysis of K⁺ currents is carried out with various ion channel blockers and voltage-clamp protocols used to remove other currents from the analysis. Most mammalian neurons contain several types of voltage-gated ion channels in contrast to squid giant axons which have only two (Bean 2007). Relatively few ionic models have been published for these cells. Determining the effect of GHK normalization of K⁺ currents in the models which are available is beyond the scope of this study.

Type 3 excitability has been observed in squid giant axons (this report; Clay 1998), bullfrog ganglion cells (Jones 1985; Jones & Adams 1987), goldfish Mauthner cells (Nakayama & Oda 2004), the original results from crustacean axons (Hodgkin 1948) and one type of ventral cochlear neurons from guinea-pigs (Manis 2003). The latter authors referred to this behaviour, i.e. a single spike in response to a sustained current stimulus, as type 2. They presented a model of this result in which the dominant current responsible for the behaviour is a rapidly activating, slowly inactivating low-threshold K⁺ current which they termed \(I_{L-T}\) (Rothman & Manis 2003a,b).

Our results raise an additional issue with regard to the HH (1952d) model of the Na⁺ current component. Specifically, the GHK equation also applies to I₉a (Vandenberg & Bezanilla 1991a). The curvature of this result is in the direction opposite to that of the \(I_{K}\) results (figure 3) because \([Na]_{o}^{+}\) >> \([Na]_{i}^{+}\). The curvature is offset by a partial, voltage-dependent block of I₉,a by extracellular calcium ions, so that I₉,a is approximately proportional to \((V−E_{Na})\) over the range of potentials spanned by the action potential (Vandenberg & Bezanilla 1991a). Consequently, normalization of peak I₉,a results during voltage-clamp steps by \((V−E_{Na})\), the procedure used by HH (1952a-d) to obtain kinetic information concerning Na⁺ channel gating, is appropriate. One result of this analysis is that the steepness of voltage activation of I₉,a closely matches that of the revised \(I_{K}\) analysis. Indeed, the revised \(n_{a}^2\) curve superimposes almost exactly with the HH \(m_{a}^2\) curve following a voltage shift (results not shown), a serendipitous finding given that the modification of \(V_{o}\) in the \(\beta_{p}\) parameter was not designed with this result in mind. This result is to be expected given the structural similarity of K⁺ and Na⁺ channels (Sigworth 2003). They differ from one another primarily in the structure of their respective permeation pathways (Hille 2001).

As noted above, the GHK normalization procedure we have used for squid K⁺ channels has recently been applied to obtain the activation curves for K⁺ channels in other preparations. The relationship of those results for excitability in cells other than squid axons is a topic for future study.

This work was supported by the Intramural Research Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland.

**APPENDIX A**

The HH (1952d) model is given by

\[
C \frac{dV}{dt} = -(I_{K} + I_{Na} + I_{L} + I_{stim}),
\]

where \(C\) is the membrane capacitance, \(C=1 \mu F \text{ cm}^{-2}\), \(V\) is the membrane potential in mV; \(t\) is the time in milliseconds; \(I_{K}, I_{Na}, I_{L}\) and \(I_{stim}\) are, respectively, the potassium ion current, the sodium ion current, the ‘leak’ current and the stimulus current, all in \(\mu A \text{ cm}^{-2}\). The \(I_{K}\) component is given by

\[
\beta_{K}m^{3}h(V, t)(V−E_{K})\]

with \(\beta_{K}=36 \text{ ms cm}^{-2}\, E_{K} = −72 \text{ mV}\) and \(dm(V, t)/dt = −(\alpha_{m}+\beta_{m})m(V, t) + \alpha_{m}\) with \(\alpha_{m} = −0.01(V + 50)/\left(\exp(0.1(V + 50))−1\right)\,ms^{-1}\) and \(\beta_{m} = 0.125\exp\left(\frac{−(V + 60)}{50}\right)\, ms^{-1}\) with \(V_{o} = 80 \text{ mV}\). The \(I_{Na}\) component is given by

\[
\beta_{Na}n^{2}(V, t)h(V, t)(V−E_{Na})\]

with \(\beta_{Na} = 120 \text{ ms cm}^{-2}, E_{Na} = 55 \text{ mV}\, dm(V, t)/dt = −(\alpha_{m}+\beta_{m})m(V, t) + \alpha_{m}\) with \(\alpha_{m} = −0.1(V + 35)/\left(\exp(0.1(V + 35))−1\right)\,ms^{-1}\) and \(\beta_{m} = 4\exp\left(\frac{−(V + 60)}{18}\right)\, ms^{-1}\), and \(dh(V, t)/dt = −(\alpha_{h}+\beta_{h})h(V, t)+\alpha_{h}\) with \(\alpha_{h} = 0.07\exp(−(V + 60)/20)\, ms^{-1}\) and \(\beta_{h} = 1/\left(\exp(−0.1(V + 30))−1\right)\, ms^{-1}\). The \(I_{L}\) component is given by

\[
\beta_{L}(V−E_{L})\]

with \(\beta_{L} = 0.3 \text{ ms cm}^{-2}\).
and $E_K = -49$ mV. The only modification in the revised model is that $V_c = 19.7$ rather than $80$ mV, which was obtained using $I_K = n(V, t)\delta q N_K \text{GHK}[V - E_K]$, as described in §3, where GHK refers to the Goldman–Hodgkin–Katz equation, $\delta$ is a constant related to the frequency of collisions of K ions with a K+ channel, $q$ is the unit electronic charge, $N_K$ is the K+ channel density and $\text{GHK}[V - E_K] = (qV/kT)\exp(q(V - E_K)/kT - 1)/(\exp(qV/kT - 1))$, where $k$ is the Boltzmann constant and $T$ is absolute temperature ($kT/q = 24$ mV at $T = 7^\circ$C). We used $I_K \sim (V - E_K)$ rather than $I_K \sim \text{GHK}[V - E_K]$ in the simulations for reasons noted in §3.

REFERENCES


