A Novel Method for the Description of Voltage-Gated Ionic Currents Based on Action Potential Clamp Results—Application to Hippocampal Mossy Fiber Boutons

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Action potential clamp (AP-clamp) recordings of the delayed rectifier K⁺ current $I_{K}$ and the fast-activated Na⁺ current $I_{Na}$ in rat hippocampal mossy fiber boutons (MFBs) are analyzed using a computational technique recently reported. The method is implemented using a digitized AP from an MFB and computationally applying that data set to published models of $I_{K}$ and $I_{Na}$. These numerical results are compared with experimental AP-clamp recordings. The $I_{Na}$ result is consistent with experiment; the $I_{K}$ result is not. The difficulty with the $I_{K}$ model concerns the fully activated current-voltage relation, which is described here by the Goldman-Hodgkin-Katz dependence on the driving force ($V-E_{K}$) rather than ($V-E_{K}$) itself, the standard model for this aspect of ion permeation. That revision leads to the second—a much steeper voltage dependent activation curve for $I_{K}$ than the one obtained from normalization of a family of $I_{K}$ records by ($V-E_{K}$). The revised model provides an improved description of the AP-clamp measurement of $I_{K}$ in MFBs compared with the standard approach. The method described here is general. It can be used to test models of ionic currents in any excitable cell. In this way it provides a novel approach to the relationship between ionic current and membrane excitability in neurons.

Keywords: rat hippocampus, action potential clamp, mathematical models, Goldman-Hodgkin-Katz, ion channels

INTRODUCTION

The action potential clamp technique (AP-clamp) is a paradigm in which an AP recorded from a neuron in current clamp is applied to that same cell in voltage clamp mode before and after the addition of a specific ion channel blocker to the external medium (Llinás et al., 1982; Bean, 2007). In this way the role of that current during an AP can be determined. The AP can also be applied computationally to a mathematical model of that current constructed from voltage step results in order to provide an additional test of the model. This approach was recently applied to $I_{Na}$ and $I_{Ca}$ in suprachiasmatic nucleus neurons (Jackson et al., 2004; Clay, 2015). In this report the method is applied to the AP-clamp recordings of Alle et al. (2009) of $I_{Na}$ and $I_{K}$ in rat hippocampal mossy fiber boutons (MFBS) at physiological temperatures ($T \approx 36 - 37^\circ$C). Those results demonstrate a significant separation in time during an AP of $I_{Na}$ and $I_{K}$, an important result for efficient neuronal signaling (Crotty et al., 2006; Alle et al., 2009; Sengupta et al., 2010). The method was
implemented using digitized results from MFBs (personal communication, Dr. H. Alle). A digitized representation of an AP from an MFB was applied computationally to the models of \( I_K \) and \( I_{Na} \) in MFBs of Engel and Jonas (2005) for model testing. The \( I_K \) AP-clamp analysis revealed a significant discrepancy between theory and experiment, which can be resolved using the Goldman-Hodgkin-Katz (GHK) equation for the fully activated current-voltage relation for \( I_K \) (Clay, 2009). The revised \( I_K \) model provides a significant improvement in the description of this component compared with a model in which a linear dependence of \( I_K \) on \( V-E_K \) was used. Computational analysis of the \( I_{Na} \) AP-clamp result (Alle et al., 2009) using the Engel and Jonas (2005) \( I_{Na} \) model was in agreement with experiment.

**MATERIALS AND METHODS**

A data set corresponding to an AP from MFBs was applied computationally to the models of \( I_K \) and \( I_{Na} \) of Engel and Jonas (2005) which are given by \( I_K = g_K n^4(V-E_K) \) and \( I_{Na} = g_{Na} m^3 h(V-E_{Na}) \), respectively, similar to the original models of \( I_K \) and \( I_{Na} \) in squid giant axons of Hodgkin and Huxley (1952), with \( g_K \) and \( g_{Na} \) constants, \( E_K \) and \( E_{Na} \) the \( K^+ \), and \( Na^+ \) reversal potentials and

\[
\frac{dx}{dt} = -(\alpha_x + \beta_x)x + \alpha_x \tag{1}
\]

with \( x = n, m, \) or \( h \), and time \( t \) in msec. The rate parameters in Equation (1) are given by Engel and Jonas (2005).

\[
\begin{align*}
\alpha_n &= -0.01(V + 55)/\exp[-(V + 55)/10] - 1; \\
\beta_n &= 0.125 \exp[-(V + 65)/80] \\
\alpha_m &= -93.8(V - 105)/\exp[-(V - 105)/17.1] - 1; \\
\beta_m &= 0.17 \exp(-V/23.3) \\
\alpha_h &= 0.00035 \exp(-V/18.7) \\
\beta_h &= 6.6/\exp[-(V + 17.7)/13.3] + 1.
\end{align*}
\tag{2}
\]

The expressions for \( \alpha_n \) and \( \beta_n \) (Equation 2) were taken from the experimental procedures of Engel and Jonas (2005). The expressions for \( \alpha_m, \beta_m, \alpha_h, \) and \( \beta_h \) (Equation 3) were taken from Supplementary Table 1 of their paper. All \( \alpha \) and \( \beta \)s in Equations (2) and (3) are in units of inverse milliseconds. The model of \( I_K \) was based on the voltage step recordings of this component in MFBs by Geiger and Jonas (2000) obtained at \( T = 34^\circ \text{C} \). It was extrapolated to \( T = 37^\circ \text{C} \) using a Q10 of 2.2 (Fohlmeister, 2015). That is, \( \alpha_n \) and \( \beta_n \) (Equation 2) were each multiplied by 1.27. The reversal potential for \( K^+ \) used in the analysis was \( E_K = -110 \text{ mV} \). \( E_K = kT/q \ln(K^+\text{aq}/K^+\text{eq}) \) where \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, \( q \) is the unit electron charge \( (kT/q = 26.7 \text{ mV} \text{ for } T = 37^\circ \text{C}) \), \( K^+\text{aq} = 2.5 \text{ mM} \) and \( K^+\text{eq} = 155 \text{ mM} \) (Alle et al., 2009)]. The recordings of \( I_{Na} \) of Engel and Jonas (2005) were obtained at \( T = 23^\circ \text{C} \). Their model of \( I_{Na} \) was extrapolated to \( T = 37^\circ \text{C} \) by multiplying each of the \( \alpha \)s and \( \beta \)s in Equation (3) by a factor of 2.8. The reversal potential for \( Na^+ \) was \( E_{Na} = 62 \text{ mV} \) (Alle et al., 2009).

The \( V \) vs. \( t \) data set \( (i = 1,2,3, \ldots ; \text{Supplementary Table 1}) \) of the MFB AP from Figure 1B of Alle et al. (2009) is represented in the top panel of Figure 1 with lines connecting adjacent points. It was applied to Equation (1) with \( x = n, m, \) or \( h \) using NDSolve in Mathematica (Wolfram Research, Inc., Champaign, IL). At \( t_1 = 0, V_1 = -80 \text{ mV} \). The initial value of \( n, n_1, \) was given by \( \alpha_n/\alpha_n + \beta_n \) with \( V = -80 \text{ mV} \) and \( \alpha_n \) and \( \beta_n \) given by Equation (2), i.e., \( n_1 = 0.1288 \). The following point of the AP data set is \( t_2 = 0.039 \text{ ms}, V_2 = -77.7 \text{ mV} \). The corresponding value of \( n, n_2 \), was determined from Equation (1) and NDSolve using \( V(t) = V_1 + (V_2 - V_1)(t - t_1)/(t_2 - t_1) \) for \( t_1 < t < t_2 \). The result was \( n_2 = 0.1289 \). More significant changes in \( n \) occur later as the membrane potential is depolarized throughout the AP. A similar analysis was applied to \( x = m \) and \( h \).

**RESULTS**

**K⁺ Current**

A digital representation of the AP-clamp recordings of \( I_K \) from Alle et al. (2009) is given in the bottom panel of Figure 1 of this report (data points with the error bars representing ± SEM, \( n = 9; \text{Supplementary Table 1} \)). These results are the differences obtained by application of the AP in Figure 1 to MFBs in voltage-clamp before and after addition to the bath of 1 mM 4-aminopyridine (4-AP), which was sufficient to completely block \( I_K \) elicited by an AP (Alle et al., 2009, 2011). They were scaled to match the \( I_K \) result in the bottom panel of Figure 1B of Alle et al. (2009). Also shown in Figure 1 is the prediction of the Engel and Jonas (2005) \( I_K \) model (dashed line; model 1) starting from the maximum level of \( I_K \) close to the peak of the AP. The rising phase of the model is not shown. These results (also given in Supplementary Table 1) correspond to \( I_{K model}(i = 1,2,3, \ldots ; \text{with } n_i \text{ determined as described above (Section Materials and Methods)}, E_K = -110 \text{ mV}, \) \( g_K = 36 \text{ mS/cm}^2 \). This model—model 1, the Engel and Jonas (2005) \( I_K \) model—does not provide a good description of the \( I_K \) AP-clamp result. The difficulty most likely concerns the fully
activated current-voltage relation for $I_K$. This result for squid axons is well described by the GHK dependence on ($V - E_K$) rather than by ($V - E_K$) itself (Clay et al., 2008), a relationship given by $I_K(n = 1) = aGHK([V - E_K])$ where $a$ is a constant, and $GHK([V - E_K]) = (qV/KT) \{exp[q(V - E_K)/KT]-1\}/[exp(qV/KT)-1]$. In their original analysis of squid axon currents Hodgkin and Huxley (1952) obtained the $I_K$ activation curve, an important result for models of $I_K$, by normalizing a family of $I_K$ records with ($V - E_K$), a linear dependence on driving force. Normalization by $GHK([V - E_K])$ should be used instead. This analysis is illustrated for $I_K$ from MFBs in Figure 2 using the results of Geiger and Jonas (2000). Their $I_K$ activation curve is shown in Figure 2 (open circles) along with a description of this result by $n^{a^4}_K(V)$ with $n^a_K(V) = \alpha_n/(\alpha_n + \beta_n)$ and $\alpha_n$ and $\beta_n$ as given above (Equation 2). Their result for $V = 50$ mV (Figure 1C of Geiger and Jonas, 2000) was not included. The $I_K$ component in squid axons is partially blocked by Na$^+$ in a voltage-dependent manner for strong depolarizations such as $V \geq 50$ mV (Bazanilla and Armstrong, 1972; French and Wells, 1977). Geiger and Jonas (2000) used an intracellular solution containing 21 mM Na$^+$. A partial block of $I_K$ in MFBs at 50 mV by this level of Na$^+$ cannot be ruled out and so this point was excluded from the analysis. The remaining results from $V = -70$ to $+30$ mV were multiplied by ($V - E_K$) to remove the linear normalization they used to obtain their result. The next step was normalization with GHK([V - E_K]) described in Clay (2009)—closed circles in Figure 2. The result is an $I_K$ activation curve that is significantly steeper than the one obtained using normalization by ($V - E_K$). A single modification in the Engel and Jonas (2005) model is sufficient to describe these results, namely a change in $\beta_n$ from 0.125 $\exp[-(V + 65)/80]$ ms$^{-1}$ to 0.125 $\exp[-(V + 65)/20]$ ms$^{-1}$, the curve labeled “$n^{a^4}_K$ revised” in Figure 2. The same modification in the original Hodgkin and Huxley (1952) model, namely replacing “80” in the exponential term of $\beta_n$ to “20,” is sufficient to describe the $I_K$ activation curve in squid axons obtained using GHK normalization of a family of $I_K$ records (Clay et al., 2008). The AP-clamp result for this version of the Engel and Jonas (2005) $I_K$ model—model 2—is given by $an^a_K GHK([V_1 - E_K])$, continuous line in Figure 1, with $a = 1.3$ mA/cm$^2$ and $n_1$ determined from Equation (1) using the modified version of $\beta_n$. This result provides a significant improvement in the description of the falling phase of the experimental record compared with model 1. The rising phase of both models underestimates the delay in rise of $I_K$ during an AP, a result that is similar to the Cole and Moore (1960) effect for voltage steps in squid axons (Discussion).

The revised $I_K$ result in Figure 1—model 2—is further illustrated by the current-voltage trajectory for $I_K$ during the AP in Figure 1 (Figure 3—dashed line). The $I_K$ gate—the $n$ variable—is maximally activated during the AP to a level of 0.283, which occurs near the latter part of the repolarization phase. The GHK current voltage relation with $n = 0.283$, also shown in Figure 3, is tangent to the trajectory at this point (arrow labeled a). The trajectory lies close to the GHK relation a considerable distance on either side of a, indicating that the time course of $I_K$ in model 2 during repolarization is largely determined by the GHK equation.

### Na$^+$ Current

Activation curve results for $I_{Na}$ from MFBs—both experimental and theoretical—taken from Engel and Jonas (2005) are shown in Figure 4 along with the revised $I_K$ results described above. The $I_K$ and $I_{Na}$ activation curves overlap almost completely (Figure 4) an observation that may be consistent with known structural similarities of voltage-gated Na$^+$ and K$^+$ channels (MacKinnon, 1991, 1995; Jan and Jan, 1997; Hanlon and Wallace, 2002).

Pooled results of AP-clamp recordings of $I_{Na}$ from Alle et al. (2009) are illustrated in Figure 5 (data points with error bars
representing ±SEM, n = 9; Supplementary Table 1). They were scaled to match the $I_{Na}$ result in the bottom panel of Figure 2B of Alle et al. (2009). The results are the differences obtained by applying the AP shown in the top panel of Figure 5 to MFBs before and after the addition of 1 μM tetrodotoxin (TTX) to the bathing medium. Also shown in Figure 5 is the prediction of the Engel and Jonas (2005) model described above, $I_{Na,i} = g_{Na,i}m_i^3h_i(V-E_{Na})$, with $i = 1, 2, 3, \ldots$ and $g_{Na,i} = 110$ nS/cm$^2$; $m_i$ and $h_i$ determined as described in Section Materials and Methods and $E_{Na} = 62$ mV (Alle et al., 2009). The model provides a good description of the experimental results. The arrow in Figure 5 highlights a slight overlap of $I_{Na}$ during repolarization in the Engel and Jonas (2005) $I_{Na}$ model attributable to an overlap of activation and inactivation. A similar result is not apparent in the experimental recordings.

The mean of the pooled results for $I_K$ and $-I_{Na}$ from Figures 1, 5, respectively, are shown in Figure 6 scaled as described in Alle et al. (2009) along with the predictions of the $I_{Na}$ model and $I_K$ model 2 described above. The arrow in Figure 6 highlights a slight overlap of $I_{Na}$ with $I_K$ during the AP, an energetically inefficient result (Alle et al., 2009).

**DISCUSSION**

This report provides further illustration of a method recently described for the analysis of ionic currents recorded with the AP-clamp technique (Clay, 2015). The work also provides an example of the utility of the GHK equation for the analysis of $I_K$ from a mammalian neuronal preparation. Traditionally, those results have been described by $I_K = g_K(V-E_K)$ with $g_K$ a constant (Hodgkin and Huxley, 1952). This expression implies, by definition, that the slope conductance for $I_K$ at a given potential below $E_K$ is the same as the slope conductance positive to $E_K$. This result is theoretically impossible when $E_K \neq 0$ because $I_K$ is proportional to $K_o^+$ with $V$ well below $E_K$ and $I_K$ is proportional to $K^+$ when $V$ is well above $E_K$. The fully activated current-voltage relation for $I_K$ outwardly rectifies, a result that is well described by the GHK equation (Clay, 2009). A similar result applies for $I_{Na}$ with a caveat. The fully activated current-voltage relation for $I_{Na}$ in squid axons in Ca$^{2+}$-free seawater is consistent with the GHK equation (Vandenberge and Bezanilla, 1991; their Figure 3). It inwardly rectifies since Na$^+$ is much greater than Na$^{2+}$. Calcium ions in normal seawater partially block $I_{Na}$ in a voltage-dependent manner with the blockade increasing as the membrane potential is hyperpolarized relative to $E_{Na}$. This effect counterbalances the inward rectification of $I_{Na}$ in the absence of divalent...
cations so that \( I_{Na} \) is approximately proportional to \( (V-E_{Na}) \) for physiological conditions over the range of potentials spanned by an AP (Vandenberg and Bezanilla, 1991). A similar mechanism may apply to \( I_{K} \) from other preparations (Worley et al., 1986; Green et al., 1987).

One consequence of the original prediction by Hodgkin and Huxley (1952) that \( I_K = g_K(V-E_K) \) is that activation curves for voltage gated \( K^+ \) channels have typically been determined by normalizing a family of \( I_K \) records using \( (V-E_K) \). An activation curve with a shallow voltage dependence is obtained (open circles, Figure 2). In contrast, normalization of those results by GHK\([(V-E_K)]\) yields an activation curve having a steepness similar to that noted by Sigworth (2003) for voltage-gated \( K^+ \) channels. Moreover, the revised \( K^+ \) channel activation curve is similar to the \( Na^+ \) channel activation curve (Figure 4). High sensitivity of these channels to voltage is important because cellular voltage changes are small (Sigworth, 2003).

Models 1 and 2 for \( I_K \) both fail to account for the delay in the rising phase of this component during an AP (Figures 1, 6), a result that is similar to the Cole and Moore (1960) effect in squid axons. Specifically, the delay in the rising phase of \( I_K \) following a voltage clamp step from relatively negative holding potentials is greater than the prediction of the Hodgkin and Huxley (1952) \( n^4 \) model (Cole and Moore, 1960). This result is significant in squid axons even for moderately hyperpolarized holding potentials such as \(-75 \text{ mV} \) (Figure 5; Clay et al., 2008). The discrepancy between theory and experiment reported here for the rising phase of \( I_K \) elicited during AP-clamp from a holding potential of \(-80 \text{ mV} \) in MFBs is a corollary of the Cole and Moore (1960) effect.

The \( I_K \) component underlying repolarization in rat hippocampal MFBs is the result of the entry of \( K^+ \) through a mixture of channels, \( Kv1, Kv3, \) and \( BK \) (Alle et al., 2011). \( BK \) channels appear not to be significant for basal APs, i.e., APs recorded under normal physiological conditions (Alle et al., 2011). The model of \( I_K \) in MFBs by Engel and Jonas (2005) is based, implicitly, on the assumption of a homogeneous population of \( K^+ \) channels. They noted that their model provided “a relatively accurate description of the voltage-dependence of activation of \( K^+ \) channels in MFBs.” This view is not necessarily at odds with the results of Alle et al. (2011) especially with regard to \( Kv \) channels that are activated rapidly. The kinetics of \( Kv1 \) and \( Kv3 \) may well be described by the same, or similar, Hodgkin and Huxley (1952) type model. In any event the falling phase of \( I_K \) obtained in AP-clamp from MFBs is consistent with a homogeneous population of \( K^+ \) channels with their fully-activated current-voltage relation described by GHK\((V-E_K)\).

The emphasis in this report is on a method, for analyzing ionic currents in neurons with an application to MFBs. The method is general. It can be applied to ionic currents in any excitable cell for which a specific blocker is available, such as TTX for \( I_{Na} \). The method requires a digitized representation of an experimentally recorded AP as well as a model of the ionic current in question obtained from voltage clamp step results such as the Hodgkin and Huxley (1952) \( m^4h \) model for \( I_{Na} \). The analysis given above for \( I_{Na} \) in MFBs is largely confirmatory of the \( m^4h \) model as given by Engel and Jonas (2005). The analysis for \( I_K \) in MFBs reveals two discrepancies between experiment and the Hodgkin and Huxley (1952) model of \( I_K \), one concerning the rising phase of \( I_K \) during an AP similar to the Cole and Moore (1960) effect and a discrepancy in the falling phase that can be accounted for by changing the fully-activated current-voltage for \( I_K \) from a linear dependence upon the \( K^+ \) driving force to the GHK dependence on \( (V-E_K) \). The method provides a complementary test of models constructed from voltage step results. An AP-clamp rapidly scans the range of membrane potentials corresponding to this waveform. In this way the GHK dependence of \( I_K \) on \( (V-E_K) \) can be elucidated for the physiological range of membrane potentials more readily than is possible with voltage steps.

The original work of Hodgkin and Huxley (1952) continues to influence the design and analysis of experiments in membrane neuroscience. The method described in this report provides a variation of their approach that can yield additional insight to the relationship between membrane excitability and the ionic currents that underlie excitability.

ACKNOWLEDGMENTS

This research was supported by the Intramural Research Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland USA. The author gratefully acknowledges Dr. Henrik Alle for providing a table of digitized results from Alle et al. (2009).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fncel.2015.00514

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**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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