A novel analysis of excitatory currents during an action potential from suprachiasmatic nucleus neurons

John R. Clay
National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

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APPROXIMATELY TEN YEARS AGO, Jackson et al. (2004) reported a comprehensive analysis of the ionic currents associated with spontaneous firing in acutely dissociated suprachiasmatic nucleus (SCN) neurons. They obtained results with voltage-clamp step recordings and with the action potential (AP) clamp technique to mathematical models. The INa result is the difference current obtained from application of the AP record to the preparation in voltage clamp for control conditions and following bath application of tetrodotoxin. Similarly, the Ica result is the difference between AP clamp results in control and following exchange of Ca2+ in the bath with Mg2+. Further details are given in Jackson et al. (2004).

METHODS

The results described in this article were obtained with Mathematica (Wolfram Research, Champaign, IL). Details of the methodology are given in RESULTS.

RESULTS

The AP from the inset of Fig. 5 of Jackson et al. (2004), along with the underlying INa and Ica components, were digitized; those records are illustrated in Fig. 1. The INa result is the difference current obtained from application of the AP record to the preparation in voltage clamp for control conditions and following bath application of tetrodotoxin. Similarly, the Ica result is the difference between AP clamp results in control and following exchange of Ca2+ in the bath with Mg2+. Further details are given in Jackson et al. (2004).

The INa component: voltage-step results. The model of INa from Engel and Jonas (2005) was the starting point of this analysis. They used the m' h gating model of INa from Hodgkin and Huxley (1952), where

\[
\begin{align*}
dm/dt &= -[\alpha_m(V) + \beta_m(V)]m + \alpha_m(V); \\
dh/dt &= -[\alpha_h(V) + \beta_h(V)]h + \alpha_h(V).
\end{align*}
\] (1)

The \(\alpha\) and \(\beta\) values in Eq. 1 used by Engel and Jonas (2005) are

\[
\begin{align*}
\alpha_m(V) &= -93.8(V - 105)/\{\exp[-(V - 105)/17.7] - 1\}; \\
\beta_m(V) &= 0.17\exp(-V/23.3); \\
\alpha_h(V) &= 0.00035 \times \exp(-V/18.7); \\
\beta_h(V) &= 6.6/\{\exp[-(V + 17.7)/13.3] + 1\}.
\end{align*}
\] (2)

The voltage \(V\) in the above equations is in millivolts, all \(\alpha\) and \(\beta\) are in units of reciprocal milliseconds, and the time \(t\) is in milliseconds. The above expressions were modified to give a qualitative description, by eye (Fig. 2A), to the INa results in Fig. 8B of Jackson et al. (2004), as well as a description of steady-state inactivation, i.e., the \(h_s(V)\) curve (Huang 1993) and the \(m_s(V)\) curve (Fig. 8C of Jackson et al. 2004), with

\[
\begin{align*}
h_s(V) &= \alpha_h(V)/[\alpha_h(V) + \beta_h(V)]; \\
m_s(V) &= \alpha_m(V)/[\alpha_m(V) + \beta_m(V)].
\end{align*}
\] (3)

The results in Fig. 2A correspond to \(I_{Ca}\) = 120 nA (data not shown) with \(h_s(V)\) in picamperes and \(V\) = -53, -48, -43, -38, -33, -28, -18, and -8 mV. Holding potential is -78 mV. The \(\alpha\) and \(\beta\) values obtained from this analysis, modified from Eq. 2, were

\[
\begin{align*}
\alpha_m(V) &= -14(V - 88)/\{\exp[-(V - 88)/17.7] - 1\}; \\
\beta_m(V) &= 0.026 \times \exp(-V/12)/8; \\
\alpha_h(V) &= 1.2 \times 10^{-4} \times \exp(-V/7)/8; \\
\beta_h(V) &= 2.3/\{\exp[-(V + 24.7)/13.3] + 1\}.
\end{align*}
\] (3)
The expressions given in Eq. 3 were used in the results that follow.

The $I_{Na}$ component: AP clamp results. The $I_{Na}$ result from the AP clamp recording (Fig. 1) was simulated using a non-traditional iterative methodology. The start point of the AP in Fig. 1 was $-56.7$ mV ($t_0 = 0$ ms, $V_o = -56.7$ mV). The initial values of $m$ and $h$, $m_0$ and $h_0$, respectively, as determined from Eq. 3 with $V = V_o$, were $m_0 = 0.076$ and $h_0 = 0.24$. The next iterative $m$ and $h$ values, $m_1$ and $h_1$, were determined from Eqs. 1 and 3 with NDSolve (Mathematica) with $V(t) = V_0 + (V - V_o)(t - t_0)(t_1 - t_0)$ for $0 < t < t_1$ ($V_1 = -56.1$ mV, $t_1 = 0.44$ ms). The results were $m_0 = 0.081$ and $h_0 = 0.24$. This procedure was continued throughout the remaining $V(t)$ vs. $t$ data set of the AP waveform for $t = 2, 3, 4, ...,$. The resulting digitized values of $I_{Na}$ were determined according to $I_{Na} = 100m_i h_i (V_i - 45)$. The latter results are illustrated in Fig. 3 below the AP along with the experimental $I_{Na}$ recording. The model provides a good description of the experiment other than a slight discrepancy near the peak of the AP (arrow in Fig. 3).

The AP clamp analysis illustrates the behavior of inactivation, $h(t)$, and activation, $m(t)$, during repolarization (Fig. 4A). Inactivation rapidly goes to completion ($h = 0$) close to the peak of the AP. Before this occurs, $h(t)$ and $m(t)$ have a non-zero overlap, indicated by the shaded area in Fig. 4A, middle, which produces a non-zero value of $I_{Na}$. If $h(t)$ went to 0 even more rapidly during this phase of the AP, the discrepancy between theory and experiment shown in Fig. 3 would be reduced or, perhaps, eliminated. Attempts to accomplish this result without modifying other aspects of the results were unsuccessful. Activation, $m(t)$, rapidly changes from 1 to 0 during the mid portion of repolarization (Fig. 4A) just before inactivation begins to recover from 0. Consequently, no additional overlap of $h(t)$ and $m(t)$ occurs throughout the remainder of the AP (Fig. 4A).

The above results are compared with a similar analysis of the Hodgkin and Huxley (1952) model with their original expressions for the $\alpha$ and $\beta$ values for $I_{Na}$ (Fig. 4B). Inactivation in their model does not go to completion during the AP. Moreover, $m(t)$ does not return to 0 as early in the AP as is the case in the SCN $I_{Na}$ analysis. For both reasons, $h(t)$ and $m(t)$ overlap considerably during repolarization, which leads to a significant $I_{Na}$ especially since the driving force for $Na^+$ increases as the membrane potential travels down the repolarization phase of the AP. Indeed, peak $I_{Na}$ during the AP is twice as large as the peak $I_{Na}$ during the upstroke phase (Fig. 4B, bottom). As a number of groups have noted, the Hodgkin and Huxley (1952) model is not energetically efficient since $I_{K}$ must overcome $I_{Na}$ during the AP to return the membrane potential to rest (Alle et al. 2009; Carter and Bean 2009; Crotty).
et al. 2006; Sengupta et al. 2010). In contrast, $I_{Na}$ flows primarily during the initial upstroke phase of the AP in mammalian neurons (Alle et al. 2009; Carter and Bean 2009), as is the case for SCN neurons (Jackson et al. 2004; Fig. 1).

An alternative model for $I_{Na}$ gating in SCN neurons. Sim and Forger (2007) have described a model of the AP from SCN neurons. The equations for $I_{Na}$ in their model were based on the voltage-step results of Jackson et al. (2004), as was the case for the model of $I_{Na}$ described above. They used $I_{Na} = 229m^2(V^{1.6} - 2)(V - 45)$ with $m = [1 + \exp(-(V + 35.2)/7.91)]^{-1}$, $n = [1 + \exp(-(V + 286)/160)]^{-1}$, and $r_{m} = 0.51 + \exp(-(V + 26.6)/7.1)$. AP clamp analysis was applied to these equations (Fig. 5). A discrepancy between theory and experiment is apparent during the latter part of the declining phase of $I_{Na}$ (arrow b in Fig. 5) similar to the results described above (Fig. 3), suggesting that this portion of the AP clamp recording is a challenge for models of $I_{Na}$. More significantly, the Sim and Forger (2007) model does not provide an adequate description of $I_{Na}$ just before and during the initial upstroke phase of the AP (arrow a in Fig. 5) in contrast to the model given in the present report (Fig. 3), which does successfully describe this portion of the $I_{Na}$ results. This portion of the $I_{Na}$ recording is important for near-threshold excitability (see Discussion).

The $I_{Ca}$ component: voltage-step results. Channel activation for $I_{Ca}$ is modeled by $r(t)$ with

$$\frac{dr}{dt} = -[\alpha(V) + \beta(V)]r + \alpha(V).$$

(4)

Inactivation is described by $f(t)$ (Tuckwell 2012). The fully activated $I_{Ca}$ current-voltage relation ($r = f = 1$) is given by the Goldman-Hodgkin-Katz (GHK) equation:

$$I_{Ca} = -C_{a}^{Ca^{2+}}P_{Ca}F_{A}(qV/kT)/[\exp(qV/kT) - 1],$$

(5)

where $C_{a}^{Ca^{2+}}$ is the extracellular Ca$^{2+}$ concentration, $P_{Ca}$ is the membrane’s permeability to Ca$^{2+}$, $F$ is the Faraday constant, $A$ is the area of the cell, $q$ is the unit electronic charge, $k$ is the Boltzmann constant, and $T$ is absolute temperature ($kT/q = 25$ mV at room temperature). The intracellular Ca$^{2+}$ concentration, $C_{i}^{Ca}$, is $\ll C_{a}^{Ca^{2+}}$. It was set to zero in the above. Equation 5 can be written simply as $I_{Ca} = aGHK(V)$, where $a$ is a constant in units of picoamperes and GHK(V) = $(V/25)/[\exp(V/25) - 1]$. (Note that $x/\exp(x) - 1 = 1$ when $x = 0$.) The peak Ca$^{2+}$ currents in Fig. 9A, top left, of Jackson et al. (2004) were divided by GHK(V) (Clay 2009). Those results are shown in Fig. 6B along with the $r_{Ca}^{Ca}(V)$ curve, where $r_{Ca}(V) = \alpha(V)/[\alpha(V) + \beta(V)]$ with $\alpha(V) = -0.048(V + 32)/[\exp(-0.13(V + 32)) - 1]$ and $f_{Ca}(V) = 0.6 \times \exp(-0.05(V + 42))$. Attempts to ascribe $I_{Ca}$ activation, $r(t)$, to a voltage-dependent process were unsuccessful. Calcium-dependent inactivation was used instead (Fox et al. 2002; Kay 1991; Luo and Rudy 1984; Stanend and Stanfield 1982). The intracellular calcium concentration, an integral part of a model of Ca$^{2+}$-dependent inactivation, was determined from $dC_{Ca}/dt = -K_{1}I_{Ca} - K_{2}C_{Ca}^{2+}$

(6)

with $K_{1} = 8 \times 10^{-5}$ M/nC and $K_{2} = 0.04$ ms$^{-1}$ (Purvis and Butera 2005). Inactivation is given by

$$\frac{df}{dt} = \frac{f_{1}(Ca^{2+}) - f(t)}{\tau_{Ca}^{in}},$$

(7)

where $K_{d} = 0.01 \mu M$ and $\tau_{Ca}^{in} = 30$ ms (Fox et al. 2002). The full model of $I_{Ca}$ is

$$I_{Ca} = ar_{Ca}^{Ca}(V)GHK(V)$$

(8)

with $r(t)$ given by Eq. 4, $f(t)$ by Eq. 7, and GHK(V) as given above. The predictions of Eq. 8 for voltage steps are illustrated in Fig. 6A for $V = -48, -38, -28, -18, -8, +2$ mV for $a = 160$ pA. Holding potential is $-78$ mV. Return potential following depolarizing steps is $-58$ mV. The records for $-18, -8, and +2$ mV are very similar to each other, as is the case experimentally (Fig. 9 in Jackson et al. 2004). [Note that $a = 160$ pA corresponds to $P_{Ca} = 4.2 \times 10^{-6}$ cm/s based on Ca$^{2+} = 1.2$ mM and a cell diameter of 10 $\mu M$ (Jackson et al. 2004)].

The $I_{Ca}$ component: AP clamp results. The $I_{Ca}$ result from the AP clamp recording (Fig. 1) was simulated as with the $I_{Na}$ result. Channel activation, $r(t)$, was determined iteratively throughout the AP record, similar to the procedure used for the $m(t)$ and $h(t)$ results described above, using Eq. 4 and NDSolve. Inactivation, $f(t)$, was also determined iteratively using NDSolve and Eqs. 6 and 7. For example, the initial conditions at the start point of the AP in Fig. 1 ($t_{0} = 0$ ms, $V_{0} = -56.7$ mV) were $C_{a}^{Ca^{2+}} = 0, f = 1$, and $I_{Ca} = -0.27$ pA. The values of $C_{a}^{Ca^{2+}}$ and $f$ at the next point of the $V_{i}$ vs. $t$ data set ($t_{1} = 0.44$ ms; $V_{1} = -56.1$ mV) as determined from Eqs. 6 and 7 were $C_{a}^{Ca^{2+}} = 0.0035 \mu M$ and $f = 1$. This procedure was continued throughout the AP record. [Inactivation remains

Fig. 5. AP clamp analysis of the Sim and Forger (2007) model of $I_{Na}$ (SF) as described in the text. The arrows a and b indicate discrepancies between their model and the experimental recording as described in the text.

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essentially unchanged until later in the AP.] The digitized values of $I_{Ca}$, $I_{Ca,j}$, are superimposed on the $I_{Ca}$ experimental recording in Fig. 7. The model provides a good overall description of the result, in particular the initial rising phase and the decay phase of $I_{Ca}$ later in the AP, although it does not accurately describe subthreshold $I_{Ca}$. The latter discrepancy is not clearly apparent in Fig. 7.

Two $I_{Ca}$ components in SCN neurons. Jackson et al. (2004) demonstrated the presence of two kinetically and pharmacologically distinct $I_{Ca}$ components in SCN neurons, a nimodipine-sensitive component and a nimodipine-insensitive component. The former is relatively small, is activated at relatively negative potentials, and has considerably faster kinetics compared with the nimodipine-insensitive component (Fig. 9 in Jackson et al. 2004).

These results were modeled as above with $r_1(t)$, the activation variable for the nimodipine-insensitive $I_{Ca}$ given by Eq. 4 with $a_{r1}(V) = -0.034(V + 35)/[exp(-0.13(V + 35)) - 1]$ and $\beta_{r1}(V) = 0.42 \times exp[-0.065(V + 45)]$. The activation variable for the nimodipine-sensitive component, $r_2(t)$, is also given by Eq. 4 with $a_{r2}(V) = -0.096(V + 42)/[exp(-0.15(V + 42)) - 1]$ and $\beta_{r2}(V) = 1.2 \times exp[-0.08(V + 54)]$. The inactivation variable was assumed to be the same for both components, i.e., the $f$ variable. The $I_{Ca}$ amplitude, parameter $a$ in Eq. 8, was 120 pA for the nimodipine-insensitive component and 38 pA for the nimodipine-sensitive component. The predictions of the model for voltage steps are shown in the Fig. 8, top, for $V = -58, -53, -48, -38, -28, -18$, and $-8$ mV. The activation curves, $r_1^2(t)$ and $r_2^2(t)$, respectively, are shown in Fig. 8, bottom, along with data points taken from Fig. 9 of Jackson et al. (2004). Further details are given in the legend of Fig. 8.

Jackson et al. (2004) provided AP clamp recordings of $I_{Ca}$ before and after addition of nimodipine to the bath (their Fig. 12). These results shown in Fig. 9 of the present article were simulated using the two-component model of $I_{Ca}$ and the procedures described above. A notable feature of the total $I_{Ca}$ result (Fig. 9A) is the inflection on the rising phase, a result mimicked by the simulations (see DISCUSSION). The AP clamp results in the presence of nimodipine are illustrated in Fig. 9B. The $r_1(t)$ component of the $I_{Ca}$ model was deleted from this analysis.

FIG. 7. AP clamp analysis of the $I_{Ca}$ model (Eqs. 5–8) as described in the text. The result of the model is shown superimposed on the experimental result. The $I_{Ca}$ model amplitude, parameter $a$ in Eq. 8, was scaled from 160 pA, the value used for Fig. 6, to 128 pA.

FIG. 8. Two components of $I_{Ca}$, as described by Jackson et al. (2004), one blocked by nimodipine (2 μM; Nim-sens) and the other one not similarly affected (Nim-insensitive). The waveforms shown at top are given by $I_{Ca} = a_{r1}(V)G_{H}H_{K}(V)$, where $t = 1$ (Nim-insensitive) or $t = 2$ (Nim-sens) as in Eq. 8, as described in the text, with $a_1 = 120$ pA and $a_2 = 38$ pA, and $r_1 = a_{r1}/(a_{r1} + \beta_{r1})$ with $a_{r1} = -0.034(V + 35)/[exp(-0.13(V + 35)) - 1]$, $\beta_{r1} = 0.42 \times exp(-0.065(V + 45))$, $a_{r2} = -0.096(V + 42)/[exp(-0.15(V + 42)) - 1]$, and $\beta_{r2} = 1.2 \times exp(-0.08(V + 54))$ for $V = -58, -53, -48, -38, -28, -19$, and $-8$ mV. Shown at bottom are the activation curves of $r_1(t)$ and $r_2(t)$, respectively. The data points are from Fig. 9 of Jackson et al. (2004), normalized by the GHK equation as described in Fig. 6 legend.

Together with AP clamp recordings are used. The traditional approach relies on voltage-step recordings of membrane current alone, as in the original analysis of Hodgkin and Huxley (1952). Models of the voltage-gated currents underlying excitability, $I_{Na}$ and $I_{K}$ in the case of squid axons, are fitted to the voltage-step results. The equations for $I_{Na}$ and $I_{K}$ obtained from this analysis are then used to simulate an AP. A comparison of simulated and experimental APs provides a test of the model. An intermediate step in this process is proposed in this report in which the model of each individual ionic component obtained from voltage-step analysis, $I_{Na}$ for example, is further tested by an experimental recording of an AP together with a recording of the respective ionic component in AP clamp. This approach may be an appropriate first step in the development of an AP model for a cell having many different voltage-gated ionic conductances, such as SCN neurons. One of the goals of this report is a realistic mathematical model of the SCN AP. The equations for $I_{Na}$ and $I_{Ca}$ described above will be used in that model.

The $I_{Na}$ component. The model of Engel and Jonas (2005) was a significant part of this analysis. Initial attempts to simulate $I_{Na}$ during an AP from an SCN neuron based on modifications of the Hodgkin and Huxley (1952) $\alpha$ and $\beta$ values for $I_{Na}$ led to a substantial $I_{Na}$ during repolarization (simulations not shown). The AP model of Engel and Jonas
(2005) predicts a separation of the $I_{\text{Na}}$ and $I_C$ components on the time axis (Clay 2013) consistent with experiment (Alle et al. 2009), which makes their model of $I_{\text{Na}}$ gating a more appropriate starting point for building a model of $I_{\text{Na}}$ gating for SCN neurons than the Hodgkin and Huxley (1952) model. The $\alpha$ and $\beta$ values for the SCN $I_{\text{Na}}$, (Eq. 3), which correspond to results obtained at room temperature (Jackson et al. 2004), are ~10 times smaller than the $\alpha$ and $\beta$ values for hippocampal mossy fiber $I_{\text{Na}}$ (Eq. 2), results also obtained at room temperature (Engel and Jonas 2005). This comparison is consistent with considerably faster $I_{\text{Na}}$ gating for the latter preparation compared with SCN neurons. Both results have been described with $m^3h$, a squid-based model (Hodgkin and Huxley 1952).

Later work found their model to be incomplete primarily on the basis of gating currents (Vandenberg and Bezanilla 1991a and other studies cited therein). Vandenberg and Bezanilla (1991b) proposed an alternative model that describes most, if not all, $I_{\text{Na}}$ results from squid axons. Their model is, unfortunately, cumbersome (not easy to use) and probably not applicable to mammalian preparations. The original $m^3h$ model is relatively simple and does describe whole cell currents from squid axons and mammalian preparations (Clay 2013). Therefore, its continued use appears to be appropriate provided the relevant $\alpha$ and $\beta$ values are also used.

As noted above, the combination of voltage-step and AP clamp results provides a more rigorous test of models of ion channel gating than voltage-step results alone. This claim is supported by the analysis of the Sim and Forger (2007) model described in Fig. 5. Their model of $I_{\text{Na}}$ developed solely from the voltage-step results of Jackson et al. (2004), provides an inferior description of the AP clamp recording of this component compared with the model of $I_{\text{Na}}$ described in this report.

The $I_C$ component. The voltage-step recordings of $I_C$ of Jackson et al. (2004), L-type $Ca^{2+}$ channel, in the absence of similar results with nimodipine do not clearly indicate the presence of two kinetically distinct $I_C$ components. In contrast, their AP clamp recording of $I_C$ in Fig. 9A is suggestive of this result, i.e., the inflection on the rising phase. This result is elucidated by the simulation in Fig. 9A. The nimodipine-sensitive component is activated at relatively negative potentials with sufficiently rapid kinetics (Fig. 8) so that it is nearly in step with the membrane potential during the upstroke phase of the AP. The nimodipine-insensitive component is activated at a slower rate, which accounts for the inflection in $I_C$ in the AP clamp recording. The model does not completely describe the secondary increase of $I_C$ following the inflection, although it does provide a good overall description of the AP clamp recording, perhaps sufficient for its use in a full model of the SCN AP. The model of $I_C$ in which this component was ascribed to a homogeneous population of channels does not exhibit an inflection in the rising phase of the current during AP clamp (Fig. 7). These results provide further evidence for the utility of the AP clamp methodology.

The fully activated current-voltage relation is an important component of models of $I_C$ regardless of channel type. A linear driving force, $(V - E_{Ca})$, for this relation, where $E_{Ca}$ is the Nernst potential for $Ca^{2+}$, is not reasonable since $E_{Ca}$ is not clearly defined, especially for a cell at rest. The Goldman-Hodgkin-Katz equation provides a sufficient description of this relation, in particular the inward rectification of $I_C$.

The simulation in Fig. 9A predicts that $Ca^{2+}$ briefly reaches the 10 $\mu$M level near the latter part of the AP (results not shown), which is sufficient to activate the $Ca^{2+}$-dependent $K^+$ channel, $I_{K,Ca}$ (Cui et al. 1997). Jackson et al. (2004) also applied the AP clamp technique to the $I_{K,Ca}$ component. The modeling approach described in this report may be useful in describing those results.

Other current components. Analysis of non-$Ca^{2+}$-activated $K^+$ currents in SCN neurons with the AP clamp technique does not appear straightforward. Jackson et al. (2004) found that addition of 10 mM TEA-Cl (TEA$^+$) to the bath completely removed net outward current during an AP. In contrast, 30 mM TEA$^+$ did not completely block the delayed rectifier $K^+$ current, $I_{K,DR}$, in voltage-step analysis (Bouskila and Dudek 1995). Perhaps modeling of both sets of results may lead to a resolution of this apparent paradox. SCN neurons also have the transient, rapidly inactivating $K^+$ current, $I_A$ (Bouskila and Dudek 1995; Itri et al. 2010), which is completely blocked by 5 mM 4-aminopyridine (4-AP) (Huang et al. 1993). Moreover, 4-AP modifies spiking behavior of SCN neurons and the shape of the SCN AP (Itri et al. 2005). These results suggest that AP clamp analysis of SCN neurons before and after bath application of 4-AP would be of interest.

Dynamic clamp: comparison with AP clamp and voltage-clamp techniques. The AP clamp technique is similar to the dynamic clamp method, an approach originally used in cardiac electrophysiology that also has been used in neuroscience and in other fields (Goaillard and Marder 2006; Wilders 2006). As the name implies, this technique involves real-time injection of current into the preparation under investigation during an ongoing experiment, a procedure not required with AP clamp. The AP clamp method does require the preparation under study to be sufficiently stable to permit several steps: 1) recording of an AP, 2) application of the AP waveform to the preparation in voltage-clamp mode in control conditions, 3) voltage-step recordings in control with a particular ionic conductance in mind, $I_{Na}$ for example, and 4) application of the AP waveform and voltage steps in voltage clamp after the addition of tetrodotoxin to the bath. The results are the differences in membrane currents in control and test conditions for both the AP and voltage steps. This report describes an extension of the method in which an experimentally recorded AP is digitized and the resulting $V_i$ vs. $t$ data set applied to mathematical models of the relevant ionic conductance, such as $I_{Na}$. The result is compared with an experimental AP clamp recording of that component either for model validation or for modification of model parameters so that the model provides a satisfactory description of both voltage-step and AP clamp measurements of the particular ionic component under investigation. A review of the literature did not yield any other reports in which this procedure had been used. A related approach concerning models of $I_{Na}$ has been reported for raphe pacemaker neurons (Milescu et al. 2008). Moreover, waveforms other than rectangular steps have been used previously. For example, Fohlmeister and Adelman (1985) used sinusoids in voltage clamp to measure $I_{Na}$ gating currents in squid giant axons, and they analyzed their results with the Hodgkin-Huxley $I_{Na}$ model. An AP waveform is, perhaps, of greater interest compared with sine waves or rectangular steps since it has direct physiological relevance. The utility of a voltage-clamp step, as shown originally by Hodgkin and Huxley (1952), is that it

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allows the investigator to change in a controlled and predetermined manner the primary independent variable of an excitable membrane, the membrane potential, and record the resulting change in the dependent variable, the net membrane ionic current. Voltage steps also, ideally, short-circuit the membrane capacitance. For these reasons they have been widely used since Hodgkin and Huxley (1952).

Summary. A novel extension of the AP clamp technique is described involving models of the ionic conductances in the cell from which the AP was recorded. The method is general. It has been applied to SCN neurons with a goal of describing a complete mathematical model of the AP in these cells. The equations for $I_{Na}$ and $I_{Ca}$ given above form one part of that model, a model that may have broad applicability not only for the SCN but also for other mammalian preparations.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

J.R.C. conception and design of research; J.R.C. analyzed data; J.R.C. interpreted results of experiments; J.R.C. prepared figures; J.R.C. drafted version of manuscript. J.R.C. edited and revised manuscript; J.R.C. approved final version of manuscript.

REFERENCES


Equation 5 in this report contains an incorrect valence, \( z = 1 \), for \( \text{Ca}^{2+} \) instead of the correct valence, \( z = 2 \). As such, results presented in Figs. 6–9 are incorrect. The author apologizes for this error and for any inconvenience associated with the publication of the article. The paper is therefore being retracted by the American Physiological Society at the request of Dr. Clay.