A Novel Analysis of Excitatory Currents during an Action Potential from

Suprachiasmatic Nucleus Neurons

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Running Head: Modeling of $Na^+$ and $Ca^{2+}$ currents during an action potential

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Abstract

A new application of the action potential (AP) voltage-clamp technique is described based on computational analysis. An experimentally recorded AP is digitized. The resulting $V_i$ vs $t_i$ data set is applied to mathematical models of the ionic conductances underlying excitability for the cell from which the AP was recorded in order to test model validity. The method is illustrated for AP's from suprachiasmatic nucleus (SCN) neurons and the underlying tetrodotoxin-sensitive $Na^+$ current, $I_{Na}$, and the $Ca^{2+}$ current, $I_{Ca}$. Voltage-step recordings have been made for both components from SCN neurons (Jackson et al. 2004). The combination of voltage-step and AP clamp results provides richer constraints for mathematical models of voltage-gated ionic conductances than either set of results alone, voltage-step results in particular. For SCN neurons the long-term goal of this work is a realistic mathematical model of the SCN AP in which the equations for $I_{Na}$ and $I_{Ca}$ obtained from this analysis will be a part. Moreover, the method described in this report is general. It can be applied to any excitable cell.

Key words: Action potential clamp, suprachiasmatic nucleus neurons, mathematical models
Introduction

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) voltage-clamp technique (Llinas et al. 1982; Doerr et al. 1989; Bean 2007). This report describes modeling of both sets of results for the Na\(^+\) current component, \(I_{Na}\), and the Ca\(^{2+}\) current component, \(I_{Ca}\), with an emphasis on AP clamp results. The latter analysis was carried out using digitized versions of AP’s in the Jackson et al. (2004) report. Those data sets were applied to the models of \(I_{Na}\) and \(I_{Ca}\) described below. This approach is, essentially, a computational application of the AP clamp technique to mathematical models. The \(I_{Na}\) analysis utilized the model of this component of Engel and Jonas (2005) from their work on hippocampal mossy fiber boutons. The \(I_{Ca}\) analysis utilized the Goldman-Hodgkin-Katz (GHK) equation for the fully-activated current-voltage relation of \(I_{Ca}\) (Goldman 1943; Hodgkin and Katz 1949; Clay 2009) and Ca\(^{2+}\)-dependent rather than voltage-dependent inactivation (Tuckwell 2012). This work demonstrates that a combination of voltage step and AP clamp results in which recordings of AP’s from the preparation under study is used in the analysis provides a useful test of models of ionic currents in excitable cells.
Methods

The results described here were obtained with Mathematica (Wolfram Research, Inc., Champaign, IL). Details of the methodology are given below.

Results

The AP from the inset of Figure 5 of Jackson et al. (2004) along with the underlying $I_{Na}$ and $I_{Ca}$ components were digitized. Those records are illustrated in Figure 1. The $I_{Na}$ 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 $I_{Ca}$ result is the difference between AP clamp results in control and following exchange of $Ca^{2+}$ in the bath with $Mg^{2+}$.

Further details are given in Jackson et al. (2004).

The $I_{Na}$ component; voltage-step results

The model of $I_{Na}$ from Engel and Jonas (2005) was the starting point of this analysis. They used the $m^3h$ gating model of $I_{Na}$ from Hodgkin and Huxley (1952) where

$$\frac{dm}{dt} = - (\alpha_m(V) + \beta_m(V))m + \alpha_m(V);$$

$$\frac{dh}{dt} = - (\alpha_h(V) + \beta_h(V))h + \alpha_h(V). \quad (1)$$

The $\alpha$'s and $\beta$'s in Equation (1) used by Engel and Jonas (2005) are

$$\alpha_m(V) = -93.8(V-105)/(\exp(-(V-105)/17.7)-1); \beta_m(V) = 0.17\exp(-V/23.3);$$

$$\alpha_h(V) = 0.00035\exp(-V/18.7); \beta_h(V) = 6.6/(\exp(-V+17.7)/3.3)+1. \quad (2)$$
The voltage $V$ in the above is in mV; all $\alpha$s and $\beta$s are in units of msec$^{-1}$. The time $t$ is in msec. The above expressions were modified to give a qualitative description, by eye (Figure 2A), to the $I_{Na}$ results in Figure 8B of Jackson et al. (2004) as well as a description of steady-state inactivation, i.e., the $h_{\infty}(V)$ curve (Huang 1993), and the $m_{\infty}(V)$ curve (Figure 8C of Jackson et al. 2004), with $h_{\infty}(V) = \alpha_h(V) / (\alpha_h(V) + \beta_h(V))$ and $m_{\infty}(V) = \alpha_m(V) / (\alpha_m(V) + \beta_m(V))$. The $h_{\infty}(V)$ and $m_{\infty}(V)$ curves are illustrated in Figure 2B. The results in Figure 2A correspond to $I_{Na} = 120 \ m^3(t)h(t)(V-45)$ with $I_{Na}$ in pA and $V= -53, -48, -43, -38, -33, -28, -18, \text{ and } -8 \text{ mV}$. Holding potential = -78 mV.

The $\alpha$s and $\beta$s obtained from this analysis, modified from Equation (2), were

$$\alpha_m(V) = -14(V-88)/(\exp(-(V-88)/17.7)-1)$$,
$$\alpha_h(V) = 1.2 \times 10^{-4} \exp(-(V+7)/8)$$,
$$\beta_h(V) = 2.3 / (\exp(-(V+24.7)/13.3)+1)$$. (3)

The expressions given in Equation (3) were used in the results that follow.

**The $I_{Na}$ component; AP clamp results**

The $I_{Na}$ result from the AP clamp recording (Figure 1) was simulated using a non-traditional iterative methodology. The start point of the AP in Figure 1 was -56.7 mV ($t_0 = 0, V_0 = -56.7$ mV). The initial values of $m$ and $h$, $m_0$ and $h_0$ respectively, as determined from Equation (3) with $V = V_0$, were $m_0 = 0.076$, $h_0 = 0.24$. The next iterative $m$ and $h$ values, $m_1$ and $h_1$, were determined from Equations (1) & (3) with NDSolve (Mathematica) and $V(t) = V_0 + (V_1-V_0)(t-t_0)/(t_1-t_0)$ for $t_0 < t < t_1$. [$V_1 = -56.1$ mV; $t_1 = 0.44$ msec.] The results were $m_0 = 0.081, h_0 = 0.24$. This procedure was
continued throughout the $V_i$ vs $t_i$ data set of the AP waveform for $i = 2, 3, 4, ...$. The resulting digitized values of $I_{Na}$ were determined according to $I_{Na,i} = 100 m^3 h_i(V_i-45)$.

The latter results are illustrated in Figure 3 below the AP along with the experimental $I_{Na}$ recording. The model provides a good description of experiment other than a slight discrepancy near the peak of the AP (arrow in Figure 3).

The AP clamp analysis illustrated the behavior of inactivation, $h(t)$, and activation, $m^3(t)$, during repolarization (Figure 4A). Inactivation rapidly goes to completion ($h=0$) close to the peak of the AP. Before this occurs $h(t)$ and $m^3(t)$ have a non-zero overlap indicated by the shaded area in the middle panel of Figure 4A 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 Figure 3 would be reduced, or perhaps, eliminated. Attempts to accomplish this result without modifying other aspects of the results were unsuccessful. Activation, $m^3(t)$, rapidly changes from 1 to 0 during the mid-portion of repolarization (Figure 4A) just before inactivation begins to recover from 0. Consequently, no additional overlap of $h(t)$ and $m^3(t)$ occurs throughout the remainder of the AP (Figure 4A).

The above results are compared with a similar analysis of the Hodgkin and Huxley (1952) model with their original expressions for the $\alpha$'s and $\beta$'s for $I_{Na}$ (Figure 4B). Inactivation in their model does not go to completion during the AP. Moreover, $m^3(t)$ 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 $m^3(t)$ overlap considerably during repolarization which leads to a significant $I_{Na}$ especially since the driving force for
\( \text{Na}^+ \) 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 (Figure 4B, bottom panel). 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 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; Figure 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 \( I_{Na} = 229 m^3(t)h(t)(V-45) \) with \( \frac{dm}{dt} = (m_{\infty} - m)/\tau_m, \frac{dh}{dt} = (h_{\infty} - h)/\tau_h \) and \( m_{\infty} = (1 + \exp(-\frac{(V+35.2)}{7.9}))^{-1}, \tau_m = \exp(-\frac{(V+286)}{160}), h_{\infty} = (1 + \exp((V+62)/5.5))^{-1}, \) and \( \tau_h = 0.51 + \exp(-\frac{(V+26.6)}{7.1}). \) AP clamp analysis was applied to these equations (Figure 5). A discrepancy between theory and experiment is apparent during the latter part of the declining phase of \( I_{Na} \) (arrow \( b \) in Figure 5) similar to the results described above (Figure 3), suggesting that this portion 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 for \( I_{Na} \) just prior to and during the initial upstroke phase of the AP (arrow \( a \) in Figure 5) in contrast to the model given in this report.
(Figure 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 (DISCUSSION).

**The $I_{Ca}$ component; voltage-step results**

Channel activation for $I_{Ca}$ is modeled by $r^2(t)$ with

$$dr/dt = -(\alpha(V) + \beta(V))r + \alpha(V).$$  

(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,

$$I_{Ca} = -Ca_{o}^{2+}P_{Ca}FA(qV/kT)/(\exp(qV/kT)-1),$$  

(5)

where $Ca_{o}^{2+}$ 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 calcium ion concentration, $Ca^{2+}$, is $<< Ca_{o}^{2+}$. It was set to zero in the above. Equation (5) can be written simply as $I_{Ca} = a \text{GHK}(V)$, where $a$ is a constant in units of pA and $\text{GHK}(V) = (V/25)/(\exp(V/25)-1)$. [Note that $x/(\exp(x)-1) = 1$ when $x=0$. The peak $Ca^{2+}$ currents in Figure 9A (left top panel) from Jackson et al. (2004) were divided by $\text{GHK}[V]$ (Clay 2009). Those results are shown in Figure 6B along with the $r^2_{\infty}(V)$ curve, where $r_{\infty}(V) = \alpha(V)/(\alpha(V) + \beta(V))$ with $\alpha(V) = -0.048(V+32)/(\exp(-0.13(V+32))-1)$ and $\beta(V) = 0.6\exp(-0.05(V+42))$. Attempts to ascribe $I_{Ca}$
inactivation, \( f(t) \), to a voltage-dependent process were unsuccessful. Calcium-dependent inactivation was used instead (Standen and Stanfield 1982; Luo and Rudy 1984; Kay 1991; Fox et al. 2002). The intracellular calcium concentration, an integral part of a model of \( Ca^{2+} \)-dependent inactivation, was determined from

\[
d\frac{Ca^{2+}}{dt} = -K_1I_{Ca} - K_2Ca^{2+} \tag{6}
\]

with \( K_1 = 3 \times 10^{-5} \) M/nC and \( K_2 = 0.04 \) msec\(^{-1} \) (Purvis and Butera 2005). Inactivation is given by

\[
d\frac{f(t)}{dt} = \left( f_1(Ca^{2+}) - f(t) \right) / \tau_{Cf},
\]

\[
f_1(Ca^{2+}) = \frac{1}{1 + \left( \frac{Ca^{2+}}{K_d} \right)^3} \tag{7}
\]

with \( K_d = 0.01 \) \( \mu \)M and \( \tau_{Cf} = 30 \) msec (Fox et al. 2002). The full model of \( I_{Ca} \) is

\[
I_{Ca} = a r^2(t) f(t) \text{GHK}(V) \tag{8}
\]

with \( r(t) \) given by Equation (4), \( f(t) \) by Equation (7) and \( \text{GHK}(V) \) as given above. The predictions of Equation (8) for voltage steps are illustrated in Figure 6A for \( V = -48, -38, -28, -18, -8, +2 \) mV for \( a = 160 \) pA. Holding potential = -78 mV. Return potential following depolarizing steps = -58 mV. The records for -18, -8 and +2 mV are very similar to each other, as is the case experimentally (Figure 9, Jackson et al. 2004).

[Note that \( a = 160 \) pA corresponds to \( P_{Ca} = 4.2 \times 10^{-6} \) cm sec\(^{-1} \) based on \( Ca_o^{2+} = 1.2 \) mM and a cell diameter of 10 \( \mu \) (Jackson et al. 2004)].
The $I_{Ca}$ component; AP clamp results

The $I_{Ca}$ result from the AP clamp recording (Figure 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 Equation (4) and NDSolve. Inactivation, $f(t)$, was also determined iteratively using NDSolve and Equations (6) & (7). For example, the initial conditions at the start point of the AP in Figure 1 ($t_0 = 0, V_0 = -56.7 \text{ mV}$) were $Ca^{2+} = 0, f = 1,$ and $I_{Ca} = -0.27 \text{ pA}$. The values of $Ca^{2+}$ and $f$ at the next point of the $V_i$ vs $t_i$ data set ($t_1 = 0.44 \text{ msec; } V_1 = -56.1 \text{ mV}$) as determined from Equations (6) & (7) were $Ca_i^{2+} = 0.0035 \mu\text{M}$ and $f = 1$. This procedure was continued throughout the AP record. [Inactivation remains essentially unchanged until later in the AP.] The digitized values of $I_{Ca}$, $I_{Ca,i} = a r^2 f_i \text{GHK}(V_i)$, are superimposed on the $I_{Ca}$ experimental recording in Figure 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 Figure 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 to the nimodipine-insensitive component (Figure 9, Jackson et al. 2004).
These results were modeled as above with $r_1(t)$, the activation variable for nimodipine-insensitive $I_{Ca}$ given by Equation (4) with $\alpha_{r1}(V) = -0.034(V+35)/(\exp(-0.13(V+35))-1)$ and $\beta_{r1}(V) = 0.42\exp(-0.065(V+45))$. The activation variable for the nimodipine-sensitive component, $r_2(t)$, is also given by Equation (4) with $\alpha_{r2}(V) = -0.096(V+42)/(\exp(-0.15(V+42))-1)$ and $\beta_{r2}(V) = 1.2\exp(-.08(V+54))$. The inactivation variable was assumed to be the same for both components, i.e., the $f$ variable. The $I_{Ca}$ amplitude, parameter $a$ in Equation (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 top panel of Figure 8 for $V = -58, -53, -48, -38, -28, -18, \text{ and } -8 \text{ mV}$. The activation curves, $r_1^2(t)$ and $r_2^2(t)$, respectively, are shown in the bottom panel of Figure 8 along with data points taken from Figure 9 of Jackson et al. (2004). Further details are given in the legend of Figure 8.

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

This report describes a novel method for testing models of ionic currents in excitable preparations in which voltage steps 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 AP’s provides a test of the model. An intermediate step in this process is proposed here 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) $\alpha$’s and $\beta$’s 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 $I_{Na}$ and $I_K$ components on the time axis (Clay 2013).
consistent with experiment (Alle et al. 2009) which makes their model of $I_{Na}$ gating a 
more appropriate starting point for building a model of $I_{Na}$ gating for SCN neurons 
than the Hodgkin and Huxley (1952) model. The $\alpha'$s and $\beta$'s for the SCN $I_{Na}$ 
(Equation 3), which correspond to results obtained at room temperature (Jackson et 
al. 2004), are approximately 10x smaller than the $\alpha'$s and $\beta$'s for hippocampal mossy 
fiber $I_{Na}$ (Equation 2), results also obtained at room temperature (Engel and Jonas 
2005). This comparison is consistent with considerably faster $I_{Na}$ gating for the 
latter preparation as compared to SCN neurons. Both results have been described 
with $m^3 h$, a squid-based model (Hodgkin and Huxley 1952). Later work found their 
model to be incomplete based primarily on 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 $I_{Na}$ results from 
squid axons. Their model is, unfortunately, cumbersome - not easy to use - and 
probably not applicable to mammalian preparations. The original $m^3 h$ model is 
relatively simple and it does describe whole cell currents from squid axons and 
mammalian preparations (Clay 2013). Therefore, its continued use appears to be 
appropriate provided the relevant $\alpha'$s and $\beta$'s 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 Figure 5. Their model of $I_{Na}$, 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 to the model of $I_{Na}$ described in this report.
The \textit{I}_{\text{Ca}} \textit{component}

The voltage-step recordings of \textit{I}_{\text{Ca}} \textit{of} Jackson et al. (2004) - L-type \textit{Ca}^{2+} \textit{channel} - in the absence of similar results with nimodipine do not clearly indicate the presence of two kinetically distinct \textit{I}_{\text{Ca}} \textit{components}. In contrast their AP clamp recording of \textit{I}_{\text{Ca}} \textit{in Figure 9A} is suggestive of this result, i.e., the inflection on the rising phase. This result is elucidated by the simulation in Figure 9A. The nimodipine-sensitive component is activated at relatively negative potentials with sufficiently rapid kinetics (Figure 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 \textit{I}_{\text{Ca}} \textit{in the AP clamp recording}. The model does not completely describe the secondary increase of \textit{I}_{\text{Ca}} 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 \textit{I}_{\text{Ca}} \textit{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 (Figure 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 \textit{I}_{\text{Ca}} regardless of channel type. A linear driving force, \((V - E_{\text{Ca}})\), for this relation where \(E_{\text{Ca}}\) is the Nernst potential for \textit{Ca}^{2+} is not reasonable since \(E_{\text{Ca}}\) 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 \( I_{Ca} \).

The simulation in Figure 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 here 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\(^+\) to the bath completely removed net outward current during an AP. In contrast, 30 mM TEA\(^+\) does 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) that 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 and voltage clamp step 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 (Wilders 2006; Goaillard and Marder 2006). As the name implies, this technique involves real-time injection of current into the preparation under investigation during an on-going 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 for voltage steps. This report describes an extension of the method in which an experimentally recorded AP is digitized and the resulting \( V_i \) vs \( t_i \) 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 to 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 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.

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Figure legends

Figure 1. Digitized representation of the AP and the underlying $I_{Na}$ and $I_{Ca}$ components in Figure 5 of Jackson et al. (2004). The results are illustrated using PlotJoined (Mathematica).

Figure 2. SCN $I_{Na}$ analysis as described in the text. A. $I_{Na}$ records from Equations (1) and (3) for $V$=-53, -48, -43, -38, -33, -28, -18, and -8 mV. Holding potential = -78 mV.

B. Inactivation, $h_{\infty} = \frac{\alpha_h(V)}{\alpha_h(V) + \beta_h(V)}$, and activation, $m_{\infty}(V)$, with $m_{\infty}(V) = \frac{\alpha_m(V)}{\alpha_m(V) + \beta_m(V)}$. Data for the inactivation curve was taken from Figure 6A of Huang (1993). The activation results were taken from Figure 8C of Jackson et al. (2004).

Figure 3. AP clamp analysis of the $I_{Na}$ model (Equations 1 & 3) as described in the text. The experimental and theoretical $I_{Na}$ results are shown superimposed below the AP. The arrow indicates a discrepancy between theory and experiment, also described in the text.

Figure 4. A. Analysis of $m^3(t)$ and $h(t)$ during repolarization of the SCN AP.

Inactivation is not complete near the initial part of this phase of the AP resulting in a non-zero overlap of $m^3(t)$ and $h(t)$ as indicated by the shaded area and the $I_{Na}$ component in the bottom panel. B. Similar analysis of the Hodgkin and Huxley (1952) model which is given by $CdV/dt = -120 \ m(t)^3h(t)(V-55) -36n(t)^4(V+72) - 0.3(V+49)$, with $C = 1 \ \mu F/cm^2$, $\alpha_m(V) = -0.1(V+35)/(\exp(-0.1(V+35))-1)$, $\beta_m(V) = 4\exp(-(V+60)/18$, $\alpha_h(V) = 0.07\exp(-(V+60)/20)$, $\beta_h(V) = 1/(\exp(-0.1(V+30))+1)$,
\[ \alpha_n(V) = -0.01(V+50)/(\exp(-0.1(V+50))-1), \text{ and } \beta_n(V) = 0.125\exp(-(V+60)/20). \]

An AP was elicited from the model by a 1 msec duration pulse having an amplitude of 10 \( \mu A/cm^2 \) (not shown). The predictions of the model illustrated here are described in the text.

Figure 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.

Figure 6. A. SCN \( I_{Ca} \) voltage-step analysis as described in the text (Equations 5-8) with \( V = -48, -38, -28, -18, -8, \text{ and } +2 \text{ mV}. \) B. Activation curve for \( I_{Ca} \ r_\infty(V) \), with

\[ r_\infty(V) = \frac{\alpha_r(V)}{\alpha_r(V) + \beta_r(V)} \text{ and } \alpha_r(V) = -0.048(V+32)/(\exp(-0.13(V+32))-1), \beta_r(V) = 0.6\exp(-0.05(V+42)). \]

The data points represent peak \( I_{Ca} \) from the voltage step recordings in Figure 9A, top panel, of jackson et al. (2004) divided by the peak current for \( V = -8 \text{ mV} \) and then divided by \( GHK(V) = (V/25)/(\exp(V/25)-1) \), as described in Clay (2009).

Figure 7. AP clamp analysis of the \( I_{Ca} \) model (Equations 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 Equation (8), was scaled from 160 pA, the value used for Figure 6, to 128 pA.

Figure 8. Two components of \( I_{Ca} \) as described by Jackson et al. (2005), one blocked by nimodipine (2 \( \mu M \)), referred to here as Nim-sensitive, the other one not similarly affected, referred to here as Nim-insensitive. The waveforms shown in the top panel
are given by \( I_{Ca} = a_i r_i^2(t) f(t) \) GHK(\( V \)), \( i = 1 \) (Nim-insensitive) or \( i = 2 \) (Nim-sens), - Equation (8), as described in the text - with \( a_1 = 120 \) pA and \( a_2 = 38 \) pA, and \( r_i = \frac{\alpha_{ri}}{\alpha_{ri} + \beta_{ri}} \) with \( \alpha_{r1} = -0.034(V+35)/(\exp(-0.13(V+35))-1) \), \( \beta_{r1} = 0.42\exp(-0.064(V+45)) \), \( \alpha_{r2} = -0.096(V+42)/(\exp(-0.15(V+42))-1) \), and \( \beta_{r2} = 1.2 \exp(-0.08(V+54)) \) for \( V = -58, -53, -48, -38, -28, -19, \) and \(-8 \) mV. Shown in the bottom panel are the activation curves, \( r_1^2(t) \) and \( r_2^2(t) \), respectively. The data points were taken from Figure 9 of Jackson et al. (2004) normalized by the GHK equation as described in Figure 6 legend.

Figure 9A. AP clamp analysis of total \( I_{Ca} \) taken from Figure 12 of Jackson et al. (2004). The simulation is as described in the text. B. Similar analysis after bath application of 2 \( \mu \)M nimodipine.