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Recovery of Cable Properties Through Active and Passive Modeling of Subthreshold Membrane Responses From Laterodorsal Tegmental Neurons

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Surkis, A., C. S. Peskin, D. Tranchina, and C. S. Leonard. Recovery of cable properties through active and passive modeling of subthreshold membrane responses from laterodorsal tegmental neurons. J. Neurophysiol. 80: 2593–2607, 1998. The laterodorsal tegmental nucleus (LDT) is located in the dorsolateral pontine reticular formation. Cholinergic neurons in the LDT and the adjacent pedunculopontine tegmental nucleus (PPT) are hypothesized to play a critical role in the generation of the electroencephalographic-desynchronized states of wakefulness and rapid eye movement sleep. A quantitative analysis of the cable properties of these cells was undertaken to provide a more detailed understanding of their integrative behavior. The data used in this analysis were the morphologies of intracellularly labeled guinea pig LDT neurons and the voltage responses of these cells to somatic current injection. Initial attempts to model the membrane behavior near resting potential and in the presence of tetrodotoxin (TTX, 1 μM) as purely passive produced fits that did not capture many features of the experimental data. Moreover, the recovered values of membrane conductance or intracellular resistivity were often very far from those reported for other neurons, suggesting that a passive description of cell behavior near rest was not adequate. An active membrane model that included a subthreshold A-type K+ current and/or a hyperpolarization-activated cation current (H-current) then was used to model cell behavior. The voltage traces calculated using this model were better able to reproduce the experimental data, and the cable parameters determined using this methodology were more consistent with those reported for other cells. Additionally, the use of the active model parameter extraction methodology eliminated a problem encountered with the passive model in which parameter sets with widely varying values, sometimes spanning an order of magnitude or more, would produce effectively indistinguishable fits to the data. The use of an active model to directly fit the experimentally measured voltage responses to both long and short current pulses is a novel approach that is of general utility.

INTRODUCTION

The laterodorsal tegmental nucleus (LDT), is located at the junction of the pons and the midbrain in the ventrolateral portion of the periaqueductal gray. Anatomic studies of this region (for review, see Leonard et al. 1995; Wainer and Mesulam 1990; Woolf 1991) indicate that the principal cells of the LDT and the adjacent pedunculopontine tegmental nucleus (PPT) are cholinergic and contain high levels of nitric oxide synthase (see Vincent and Kimura 1992). Conncetional studies indicate that these neurons give rise to important ascending projections, including a major cholinergic input to the thalamus (Hallanger et al. 1987; Mesulam et al. 1983; Satoh and Fibiger 1986; Sofroniew et al. 1985; Woolf and Butcher 1986). These findings support the concept of an ‘‘ascending cholinergic reticular system,’’ which first was proposed by Shute and Lewis (1967). LDT and PPT cholinergic neurons also have local and descending projections that include a projection to a region of the medial pontine reticular formation (Jones 1990; Mitani et al. 1988; Semba 1993) from which rapid eye movement (REM) sleep can be induced at short latency after microinjections of cholinergic agonists (Baghdoyan et al. 1987; Quattrochi et al. 1989; Vanni-Mercier et al. 1989). Thus these mesopontine cholinergic neurons have a pattern of projections that suggests a functional role in the modulation of global behavioral states.

Several lines of physiological evidence support this view and further indicate that mesopontine cholinergic neurons function specifically in the generation of the electroencephalographic (EEG)-desynchronized states of waking and REM sleep (for review, see Jones 1991; Steriade and McCarley 1990). Single-unit recording studies in cat (El Mansari et al. 1989; Steriade et al. 1990a) and rodent (Kayama et al. 1992) have identified several subpopulations of putative cholinergic neurons within the PPT and LDT. Some cells fulfill the role of REM-on cells, having their highest firing rates during REM sleep and lowest during slow-wave (SW) sleep. Others display high firing rates in both waking and REM sleep and have lower rates during SW sleep. Still others, observed in cat, fire phasically with PGO waves in REM sleep (Steriade et al. 1990b). Thus mesopontine cholinergic neurons appear to be a functionally heterogeneous group of cells the firing of which is related to EEG-desynchronized states. The key factors underlying this heterogeneity are not clear, although they are likely to derive from differences in membrane properties, morphology, and synaptic input signals. To begin to evaluate the relative importance of these factors, we have undertaken a quantitative analysis of the cable properties of principal LDT neurons. This analysis is a necessary step toward producing biologically realistic models of these cells. Such models can be used to evaluate the factors generating cell output and can be useful tools for studying the interactions between mesopontine cholinergic neurons and other sleep-related neurons.

In studying cable properties, the question of the nature of
the integration of neuronal input must be considered. Although the classic concept of dendrites as passive integrators (for review, see Rall et al. 1992) has been increasingly overshadowed by a growing body of evidence demonstrating the presence of active conductances in dendrites (for review, see Johnston et al. 1996; Mel 1994), the passive model often still is applied to behavior in selected voltage regimes. The approach taken here was to conduct two analyses of the subthreshold membrane properties, one in which the membrane was assumed to be passive and one in which it was assumed that voltage-dependent conductances were contributing to cell behavior. Detailed information on cell geometry was used in each of these analyses; the cells analyzed in this study are a subset of those analyzed in a previous morphological study (Surkis et al. 1996) that concluded that the irregularity of the dendritic geometry of these cells precluded the use of equivalent cylinder simplifications (Rall 1977) in analyzing their membrane properties. Through a comparison of the results of each of these approaches, it was seen that the subthreshold behavior of LDT cells could not be adequately described by a passive membrane model.

METHODS

Experimental methods

The data analyzed in this study was culled from a subset of cells analyzed in our previous morphological study (Surkis et al. 1996). Briefly, sharp electrode intracellular recordings were obtained from a brain stem slice preparation made from female Hartley guinea pigs (obtained from Hilltop Labs; 175–300 g). The recordings analyzed in this study were all made in standard Ringer at 33°C and containing (in mM) 124 NaCl, 5 KCl, 1.2 NaH2PO4, 2.7 CaCl2, 3 MgSO4, 26 NaHCO3, 10 glucose, and 0.5–1.0 μM tetrodotoxin (TTX; Sigma). Data were acquired with a digital computer using either pClamp or Axotape software (Axon Instruments). Both long (300 ms) and short (1 ms) hyperpolarizing current pulses were applied to these cells. The voltage response to the long current pulse was recorded at 1–2 kHz, and the short pulse response was recorded at 25 kHz. Both the long and short current pulse protocols were run 50 times, and the voltage data saved was the average over those 50 responses. The short current pulse protocol was run from the resting membrane potential. The long current pulse protocol was run either from the resting membrane potential or from a level ±10 mV depolarized to the resting membrane potential, to minimize the visible sag in the voltage response. Additionally, a series of current pulse protocols was applied to the cells to characterize their physiology with respect to the physiologically identified subgroups of LDT cells, as described previously (Leonard and Llinás 1990, 1994; Surkis et al. 1996).

During the intracellular recording, neurons were injected with biocytin, visualized for tracing with diaminobenzidine and osmicated to reduce shrinkage and intensify staining (Ornung and Ulfhake 1990). Details of the histological processing and cell reconstruction were described previously (Surkis et al. 1996). In that study, some reconstructions were obtained from slices that were resectioned and some were obtained from slices that were whole mounted. All reconstructions used in this study were obtained from osmicated and whole-mounted sections. Neurons visualized with this procedure were traced and computer reconstructed using the neuron tracing system (Sun Technologies) (Capowski 1985). The soma and dendrites of cells were traced using 700–1,700 points, where each point contained information about diameter and relative three-dimensional coordinates of position. The interval between traced points was typically between 2 and 4 μm. Neurons were observed with ×100 oil-immersion lens during the tracing process, using a total system magnification of 1,250. No corrections were made for shrinkage (Surkis et al. 1996).

Computational methods

DATA FILTERING. The only filtering applied to the data before analyzing it was to discard a portion at the beginning of the response that was assumed to be contaminated by the capacitative transient of the electrode. Two methods were used to obtain an estimate of the degree of contamination of the data from this source. After a recording on a cell was completed, the electrode was withdrawn until it was just outside the cell, and then current pulses were applied and the capacitative transients were measured. A second method was to discard varying portions of the data, fit the remaining trace, and compare the extracted parameters, with the assumption that removing a small segment of data that was contaminated by the capacitative transient would cause a marked change in the extracted parameters. The first method was applied to one cell and the second to several cells in preliminary analyses. Both methods for artifact determination are problematic, so the results were used to make a general estimate of data to be discarded that was applied across all the cells analyzed. The first 2 ms of all the long pulse responses and the first 4 ms of all the short pulse responses were discarded.

PASSIVE MEMBRANE MODEL. In analyses where a passive membrane was assumed for subthreshold behavior close to the resting membrane potential, cell behavior was described using the generalized cable equation, where variations in dendritic diameter were allowed (Rall 1962), as follows

\[ C_m \frac{\partial i}{\partial t} + G_m(v - E_m) + G_{ax}(v - E_{ax}) = \frac{a}{2R} \left( \frac{\partial^2 v}{\partial x^2} + \frac{\partial}{\partial x} \frac{\partial a}{\partial x} \right) \left[ 1 + \left( \frac{\partial a}{\partial x} \right)^2 \right]^{-1/2} \]

where \( C_m \) is membrane capacitance, \( G_m \) is membrane conductance, \( E_m \) is the membrane conductance reversal potential, \( R_i \) is axoplasmic resistivity, \( a \) is dendritic radius, \( x \) is distance along a process, and \( v \) is membrane potential. The shunt conductance term, \( G_{ax} \), with reversal potential, \( E_{ax} \), is only nonzero at the soma because the shunt is assumed to be due to the presence of the intracellular electrode.

A numerical solution to the equation was implemented using spatial differencing and backward Euler in time. Sealed end boundary conditions were assumed at the dendritic terminals, and current conservation and voltage continuity were used to define conditions at branch points. Intracellular resistivity, membrane conductance, and membrane capacitance were all assumed to be spatially uniform. In the numerical implementation, the spatial grid was uniformly spaced in each dendritic segment, and diameters at each point were determined by linear interpolation between the actual traced points. The number of spatial nodes in the integration was generally between 200 and 600. The temporal integration step was 0.04 ms for the short pulse response and 0.25 ms for the long pulse response.

ACTIVE MEMBRANE MODEL. When it was assumed that voltage-dependent conductances were contributing to subthreshold membrane properties, cell behavior was described using the Hodgkin Huxley formalism (Hodgkin and Huxley 1952), where the active currents assumed to be present were a transient subthreshold K+ current, the A current (Sanchez et al. 1998), and/or a cation current that has a depolarizing influence and is activated on hyperpolarization, the H current (Leonard and Llinás 1994; Rainnie et al. 1994).
A third subthreshold current that would be present in these cells is a persistent Na⁺ current (Leonard and Kumar 1993), but because the analyzed recordings were made in TTX, this current was eliminated. The cable equation is the same as that used for the passive model, with the addition of the terms describing the voltage-dependent conductances. The equations are as follows

\[
C_m \frac{\partial v}{\partial t} + G_m(v - E_m) + G_h(v - E_h) + G_m h(v - E_m) + G_m m(v - E_m) = \frac{a}{2R} \left[ \frac{\partial v}{\partial x} \right]^2 + \frac{\partial}{\partial x} \left[ \frac{\alpha}{\beta} \left( \frac{\partial v}{\partial x} + \frac{\partial v}{\partial t} \right) \right] + \left( \frac{\alpha}{\beta} \right)^2 \right]^{-1/2} \right] \tag{1}
\]

\[
\frac{\partial m}{\partial t} = \frac{m_{\infty} - m}{\tau_m} \tag{2}
\]

\[
\frac{\partial h}{\partial t} = \frac{h_{\infty} - h}{\tau_h} \tag{3}
\]

\[
\frac{\partial m}{\partial t} = \frac{m_{\infty} - m}{\tau_m} \tag{4}
\]

where \( G_m \) and \( G_h \) are the maximum conductances for the H current and the A current, respectively, and \( E_m \) and \( E_h \) are the H current and potassium reversal potentials, respectively. The variables in Eqs. 2–4 describe the voltage dependence and kinetics of the A current and H current; \( m_{\infty} \) and \( h_{\infty} \) are the activation and inactivation parameters for the A current, and \( m \) and \( h \) is the H-current activation parameter. In Eq. 2, \( m_{\infty} \) and \( \tau_m \) are the steady-state value and time constants for A-current activation, with analogously defined steady-state values and time constants for A-current inactivation and H-current activation in Eqs. 3 and 4, respectively. The shunt term in Eq. 1 is only nonzero at the soma, as in the passive case.

Parameters for H current, as follows, were taken from values for thalamic relay neurons (Huguenard and McCormick 1992; McCormick and Pape 1990)

\[
m_{\infty} = \frac{1}{1 + \exp \left[ \frac{(v + 75)}{5.5} \right]}
\]

\[
\tau_m = \frac{1}{\exp[-14.06 - 0.86v] + \exp[-1.87 + 0.070v]}
\]

The value for the H-current reversal potential was taken to be -40 mV, based on the thalamic relay neuron data. The following parameters, used to model the A current, were taken from a study done on LDT cells in our laboratory (Sanchez et al. 1998)

\[
m_{\infty} = \frac{1}{1 + \exp \left[ \frac{(v + 39)}{-5.6} \right]}
\]

\[
h_{\infty} = \frac{1}{1 + \exp \left[ \frac{(v + 57)}{4.8} \right]}
\]

\[
\tau_m = 0.7 \text{ ms}
\]

\[
\tau_h = 18 \text{ ms}
\]

The A-current activation and inactivation parameters were determined from whole cell voltage-clamp experiments and were corrected for errors due to access resistance of the recording electrode and cable structure of the neuron (Sanchez et al. 1998). The amplitude and kinetics also were adjusted to physiological temperature levels (Huguenard et al. 1991) because the whole cell recordings were made at room temperature. The value for the A-current reversal potential (assumed to be the potassium reversal potential) was taken as -97 mV based on measurements in LDT neurons (Leonard and Llinás 1994).

As for the passive model, a numerical solution to the cable equation was implemented using spatial differencing and backward Euler in time. A split Euler method was used for the simultaneous solution of all the equations. Backward Euler was used in the solution of Eqs. 2–4, where the values for \( m_{\infty}, h_{\infty} \), and \( m \) were calculated using the value of \( v \) determined in the previous solution of Eq. 1 (Mascagni 1989). Boundary conditions, assumptions of spatial uniformity, and spatial and temporal grid sizes were all the same as was described for the implementation of the passive model.

PARAMETER EXTRACTION PROTOCOL. Whether the active or passive model was used to describe the cell behavior, the general procedure that was used to determine the membrane parameters was the same. The membrane parameters of a particular cell were chosen to be those which produced a solution to the preceding equation(s) that provided the best fit to the recorded voltage response to current injection at the soma. For each parameter set, the fits were calculated to voltage responses to both long and short current pulses. The parameter space was searched using a simplex optimization algorithm (Nelder and Mead 1965; Press et al. 1988), which was implemented using the C programming language. Because voltage responses of different lengths were fit simultaneously, the error function was calculated by taking the sum of squared errors between the recorded and calculated data for each trace, dividing it by the number of data points in that trace, and summing the errors across traces.

For any given cell, the simplex optimization algorithm was run multiple times, where runs differed based on the values that were assigned to certain fixed parameters. A given simplex run generally took between 10 and 100 h running on a Sparc 10, Sparc 20, or IBM RS6000. Parameters thus could be determined in one of two ways. Some parameters were determined directly by the simplex optimization algorithm, where the only constraint on the value that a parameter could take on was that it be nonnegative. Other parameters, which were fixed for any given simplex run, were chosen based on a comparison of goodness of fit across the different runs of the simplex. These parameters were fixed at a relatively small number of values, generally between 5 and 10. The values they were assigned to were chosen to be within a physiologically plausible range, and the interval between values was based on the sensitivity of the simplex outcome to changes in that parameter. Due to computational constraints, it was not possible to execute simplex runs for every possible combination of the values chosen for each fixed parameter. The simplex was run for various parameter combinations until the parameter space was mapped out to the extent that, based on assumptions about the smoothness of the parameter space, certain areas of the space could be mapped out in more detail.

The parameters to be determined in either of the ways described above depended on which model was used to describe the cell behavior. Whether the active or passive model was used, the values of \( C_m, G_m, G_h, \) and \( G_{sh} \), always were varied freely, where \( G_{sh} \) and \( G_h \) are the somatic shunt during the recording of the long and short pulse responses, respectively. All parameters other than the shunt parameter are assumed to be the same throughout the course of the experiment. Shifts in the shunt values have been observed over the course of recordings, and because there may be a significant time interval between the long and short pulse recording protocols, two parameters are used for the shunt to allow for changes between the recording of the long and short current pulse responses. In addition to the above parameters, when the passive model was used, the value of \( R \) was varied freely in some cases. In other cases, and when the active model was used, \( R \) was determined by fixing it at a number of values, as described above. When the active model was used, the conductance parameters, \( G_a \) and
also were varied freely. However, for most cells only one of these conductance parameters was included; for cells where there was no apparent H current and the best fitting $G_H$ parameter was consistently negligible, the H current was eliminated from the fitting, and, similarly, the A current was eliminated from the fitting in cases where it appeared to play an insignifican role. In all cases, $E_m$ and $E_{sh}$ were fixed variables that were set to a number of different values. When the active model was used, there were two additional variables relating to the active conductances which are determined by this method. The first is $\Delta m$, as shown below

$$m_{\Delta m} = \frac{1}{1 + \exp\left[\frac{v - (75 + \Delta m)}{5.5}\right]}$$

which represents the shift in the voltage at which half-maximum of the steady-state value for H-current activation is reached. The second variable is $\Delta A$, as shown below

$$m_{\Delta A} = \frac{1}{1 + \exp\left[\frac{v - (79 + \Delta A)}{5.6}\right]}$$

which represents the shift in the voltage dependence of the A-window current.

**ERROR EVALUATION.** Once a parameter set was determined with the above fitting procedure, an average percentage error (PE) was calculated. This PE was calculated as follows. Two sums of squared residuals (SSR) were calculated: one between the model-generated and the experimentally recorded voltage responses and one between the experimental traces and generated voltage traces that provided a “perfect” fit to the experimental data. These perfect fits were found by fitting either the full experimental traces or subsections of these traces to sums of exponentials and/or lines so that the fit to the whole trace was perfect to the eye; the SSR between these fits and the experimental traces were taken as a measure of noise-only error. This difference between the noise-only SSR and the SSR between experimental and model-generated traces provides a measure of model-only error (assuming no correlation between noise and model error). The square root of each of the model-only errors was divided by the root mean square (RMS) of the experimentally recorded voltage trace. These ratios of the RMS of model error to the RMS of the data were used as a measure of percentage model error per trace and were averaged across short and long pulse responses. The average was taken either across those traces that were used in the fitting procedure or across an expanded data set, which included several additional voltage responses. The additional voltage traces were the responses to current pulses of different amplitudes, recorded during the same experimental protocols as the voltage responses used in the fitting procedure.

**RESULTS**

The cells analyzed here are a subset of the cells analyzed in a previous morphology study (Surkis et al. 1996). Of the 11 cells that were included in that morphology analysis, 5 were analyzed in this study. In addition to satisfying the criteria for complete fills and matching the physiological properties characteristic of LDT type II neurons (Leonard and Llina 1990, 1994; Surkis et al. 1996), these 5 cells were all reconstructed from whole mounts and had complete data available from both the long and short current pulse protocols in TTX. These cells had an average input resistance of $92 \pm 13$ (SD) MΩ (range: 80–108 MΩ) and an average resting membrane potential of $-63 \pm 8$ mV (range: $-54$ to $-72$ mV). The reconstructions of each of the five cells are shown in Fig. 1, A–E.

**Cable parameter extraction with passive membrane model**

Initially, the voltage responses from the five cells analyzed were examined to see whether they satisfied the requirement of linearity for a passive membrane model. The steady-state I-V curves for the long pulse voltage responses appeared quite linear (Fig. 1F), whereas voltage responses that were scaled by the value of the corresponding current pulses and then overlaid revealed slight deviations from linearity (Fig. 1G). The question of what was sufficiently linear was deferred, and rather a fit was attempted to the data using only a passive model to see how well this model was able to reproduce the cell behavior.

When the parameter extraction was done using a passive membrane model, membrane conductance ($G_m$), membrane capacitance ($C_m$), the values of the somatic shunt during the each of the recording protocols ($G_{sh}$) and, in some cases, the intracellular resistivity ($R_i$) were varied freely. The reversal potentials for the shunt ($E_{sh}$) and leakage ($E_{leak}$) conductances were fixed at several different values, and, in some cases, $R_i$ was held fixed at values between 200 and 1,000 Ω·cm. The simplex optimization algorithm was run with either the reversal potentials for the shunt conductance and leakage conductance both held fixed at the resting membrane potential, with the shunt reversal potential depolarized to the resting membrane potential and the leakage reversal potential hyperpolarized to it, or with the leakage reversal potential depolarized to the resting membrane potential and the shunt reversal potential hyperpolarized to it.

The results of the parameter extraction done assuming a purely passive membrane were problematic, producing fits to the short and long current pulse responses that did not consistently capture the major features of the data. The fits for those cells that had visible sag back in the voltage traces (cell 518 and cell 815) are shown in Fig. 2. The fits for those cells without visible sag back are shown in Fig. 3. When the PE was calculated for only those traces that had been used in the fitting, the values ranged from 3.3 to 81.1% (mean = 4.7 ± 2.0). Even in the cases where the fits produced were good, however, the parameters that produced these fits indicated a problem with the passive model. For one cell, the best fit was found with a value of membrane...
capacitance $\geq 2 \mu F/cm^2$ and an intracellular resistivity value of 2,800 $\Omega$-cm, and for another the best fit was achieved with a membrane capacitance of 1.6 $\mu F/cm^2$ and an intracellular resistivity value of 2,200 $\Omega$-cm. These values are far outside the range of values reported for other cells and indicate that the passive model is not adequate for explaining the cell behavior. For another cell, the sets of cable parameters that produced the best fits to data did so with values of the

- FIG. 1. Reconstructions of analyzed cells and a linearity test of voltage responses. A: cell 225. B: cell 518. C: cell 526. D: cell 810. E: cell 815. F: steady-state I-V curves of recorded neurons were fit well by a line as shown for cell 526. G: scaling the voltage responses by the corresponding current pulse amplitudes showed that the traces overlaid fairly well, but not perfectly, even within the noise constraints.

- FIG. 2. Optimal fits found to data using a passive membrane model for those cells which exhibited sag-back. Parameters shown are those used to calculate these optimal fits. A1: fits to voltage responses to 1-ms current pulses for cell 518. A2: fits to voltage responses to 300-ms current pulses for cell 518. B1: fits to voltage responses to 1-ms current pulses for cell 815. B2: fits to voltage responses to 300-ms current pulses for cell 815.
FIG. 3. Optimal fits found to data using a passive membrane model for those cells which did not exhibit sag-back. Parameters shown are those used to calculate these optimal fits. A1: fits to voltage responses to 1-ms current pulses for cell 225. A2: fits to voltage responses to 300-ms current pulses for cell 225. B1: fits to voltage responses to 1-ms current pulses for cell 526. B2: fits to voltage responses to 300-ms current pulses for cell 526. C1: fits to voltage responses to 1-ms current pulses for cell 810. C2: fits to voltage responses to 300-ms current pulses for cell 810.

membrane conductance that ranged from $3 \times 10^{-5}$ to 43 $\mu$S/cm$^2$. The range of values is so large that, in this case, the method proves to be effectively useless in determining the parameter value. The parameters resulting from the above analyses are shown in Table 1. It should be noted, that arbitrary combination of parameters within the parameter ranges discussed here and detailed in Table 1 will produce effectively indistinguishable fits; only specified combinations will produce those best fits. Therefore, in addition to the ranges, those parameter sets with a PE within 2% of the minimum PE for a cell are shown in italics in Table 1. The medians of those parameter sets was determined for each cell.

<table>
<thead>
<tr>
<th>Cell</th>
<th>$R_c$, $\Omega$-cm</th>
<th>$C_m$, $\mu$F/cm$^2$</th>
<th>$G_m$, $\mu$S/cm$^2$</th>
<th>$G_{sh}$, nS</th>
<th>$E_l$, mV</th>
<th>$E_{sh}$, mV</th>
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</thead>
<tbody>
<tr>
<td>Cell 225</td>
<td>200–600</td>
<td>0.91–1.01</td>
<td>0.00003–43</td>
<td>7–16</td>
<td>72–75</td>
<td>70–72</td>
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<tr>
<td></td>
<td>400</td>
<td>0.99</td>
<td>43</td>
<td>7/9</td>
<td>75</td>
<td>70</td>
</tr>
<tr>
<td>Cell 518</td>
<td>2,200</td>
<td>1.61</td>
<td>68</td>
<td>1/3</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Cell 526</td>
<td>1,800–4,200</td>
<td>2.11–2.17</td>
<td>13–120</td>
<td>5–15</td>
<td>60–75</td>
<td>60–75</td>
</tr>
<tr>
<td></td>
<td>2,800</td>
<td>2.11</td>
<td>50</td>
<td>11/10</td>
<td>60</td>
<td>75</td>
</tr>
<tr>
<td>Cell 810</td>
<td>800–1,700</td>
<td>1.08–1.13</td>
<td>12–40</td>
<td>4–9</td>
<td>60</td>
<td>65–70</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>1.08</td>
<td>12</td>
<td>9/8</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Cell 815</td>
<td>200–1,000</td>
<td>1.26–1.46</td>
<td>116–122</td>
<td>0–10</td>
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<td>1.46</td>
<td>122</td>
<td>10/0</td>
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<td>10/0</td>
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</table>

Ranges in parentheses are negative numbers. Italicized figures indicate parameter sets within 2% of the set producing the minimum PE. The two numbers indicated for individual $G_{sh}$ values denote $G_{sh,l}$ and $G_{sh,s}$, respectively. Note that cell 518 had only one parameter set with a PE <10%.
Cable properties of LDT neurons

Cable parameter extraction with active membrane model

Because of the problems with the results of the passive membrane parameter extraction protocol, we concluded that the passive model did not provide an adequate description of the cell behavior. The most obvious correction to make was to expand the model to include active conductances. The active conductances that are the most likely to contribute to the subthreshold membrane behavior are the transient subthreshold K⁺ current, the A current (Sanchez et al. 1998), an anomalous rectifying cation current, the H current (Leonard and Llinás 1994; Rainnie et al. 1994), and a persistent Na⁺ current (Leonard and Kumar 1993). Because the recordings analyzed were made in TTX, only the first two currents were included in the model. The parameter extraction protocol using the active model was run on the five cells discussed above. Before using this new protocol on the recorded data, it was tested using voltage traces produced by the NEURON software package (Hines 1989); these analyses are discussed in the Appendix.

The expanded model introduced two additional parameters to be determined with the simplex optimization algorithm, the maximum H- and A-current conductance amplitudes (G_H and G_A, respectively). Because of the increased number of parameters and the relatively weak dependence of the results on the value of the intracellular resistivity, R_i, always was held fixed. The voltage dependence of the A-current window, Δ_A, and the voltage dependence of H-current activation, Δ_H, also were held fixed for each run of the simplex optimization algorithm. Also, as in the passive case, the values of E_m and E_sh were fixed at a number of values, where a range of values both above and below the resting membrane potential was tried for each of these parameters.

The simplex optimization algorithm was run for a limited number of combinations of the five fixed parameters discussed due to computational constraints. Initially the fitting procedure was run for fixed pairs of E_m and E_sh where E_m was assumed to be between −70 and −55 mV and E_sh was assumed to be between −30 and −80 mV. These combinations of E_m and E_sh were run for a number of different fixed values of Δ_H, Δ_A, and R_i. Values of Δ_H and Δ_A varied from −35 to 35 mV, and values of R_i ranged from 200 to 1,000 Ω·cm. Then Δ_H and/or Δ_A (depending on which currents are present in that cell) are varied for those values of E_m and E_sh that produced the best fits, with R_i remaining fixed at a single value. Finally, R_i is varied for those values of E_m, E_sh, Δ_H, and/or Δ_A that produced the best fits. The actual procedure was generally done in a more iterative manner than the idealized version described here, with the size of the intervals between fixed values varying based on individual cell sensitivities. Figure 4 illustrates this procedure for cell 815. In Fig. 4A it can be seen that the lowest values of PE are obtained for a shunt reversal potential of −55 mV and a membrane conductance reversal potential of −60 to −70 mV; in this figure, the PE values shown are the minimum across all values of Δ_H and R_i. Figure 4B then shows PE as a function of Δ_H for two of the combinations of E_m and E_sh that produced the best fits; in this figure, the PE is the minimum across all values of R_i. Figure 4C then shows the PE as a function of R_i for the shunt and leakage reversal potentials fixed at the values shown in Fig. 4B and Δ_H fixed at those values that provided the lowest PE for each of the reversal potential combinations.

While the additional conductances in the active model increase the number of parameters to be determined, they also expand the voltage range over which the model is expected to provide a good fit to recorded data. Therefore rather
than computing the PE for only the fit traces, as reported for the passive fits, the PE considered here was calculated for the fit traces along with an additional three to five long pulse responses and, in some cases, an additional short pulse response. In this way, rather than only evaluating how the additional parameters might improve the fit over the passive case, the model is tested to see how well it accounts for the behavior outside the fit regime.

The use of the active rather than passive model in the parameter extraction protocol resulted in a decrease in PE in all cases. The minimum PE calculated for those traces that were used in the fitting procedure ranged from 2.7% for cell 518 to 5.2% for cell 815. These values represented an average decrease of 17% in the PE from the passive case (range: 3–36%). The PE calculated for the full set of voltage traces, including those not used in the parameter extraction, ranged from 2.3% for cell 810 to 4.2% for cell 815. When the PE to the full set of traces was calculated for the passive model results, these values ranged from 3.3% for cells 225 and 526 to 9.2% for cell 815. The average reduction in PE was 26% (range: 9–54%). The differences in the PE for the active and passive models are summarized in Fig. 5. These changes in PE were, however, not always large, and the utility of the active membrane methodology was more clearly evident in the other improvements seen: the sizes of the ranges of effectively indistinguishable parameters were often significantly smaller than in the passive case, and the values spanned by these ranges were nearly all within the bounds of parameters reported for other cells.

The sizes of the range of effectively indistinguishable values of $C_m$, $R_i$, and $G_m$ for cells 225, 518, and 810 were almost all decreased when the active model was used. While the range of acceptable values for membrane conductance found assuming a passive model had varied over six orders of magnitude for cell 225 and one order of magnitude for cell 518, when the parameter extraction was done using the active model, the ranges were decreased to a factor of ~2 in both cases. For cells 526 and 815, the sizes of the parameter ranges were not decreased, but most of these ranges were shifted from the values determined in the passive case, which had been outside the ranges reported in the literature for other cells. The value determined for the intracellular resistivity for cell 526 using the passive model was 2,200 $\Omega\cdot$cm, whereas the active model produced a range of values from 400 to 800 $\Omega\cdot$cm. The membrane conductance range determined for cell 815 was 116–122 $\mu$S/cm$^2$ for the passive model, but decreased to 24–89 $\mu$S/cm$^2$ for the active model. The average parameter values across cells were determined as for the passive case. Those parameter sets with a PE within 2% of the minimum for a given cell were evaluated to determine the median parameter set (it was observed that the parameter set that had the median value for any of the parameters had the median value for all the parameters). Those five resultant parameter sets then were averaged to determine an average across cells for each parameter value. These average values are the following: $R_i = 600 \pm 200 \Omega\cdot$cm; $G_m = 39 \pm 19 \mu$S/cm$^2$; $C_m = 1.14 \pm 0.11 \mu$F/cm$^2$.

The parameter ranges determined using the active membrane model and the parameter sets with PE within 2% of the minimum PE for each cell are shown in Table 2.

When including the active conductances in the model, it was found that for three of the five cells the results were improved when the A current was added to the model but that the addition of the H current resulted in no improvement. These cells (cells 225, 526, and 810) had no visible sag back in the traces fit and little or no visible sag back in the full set of voltage traces. The improvements in fits achieved using the active model versus the passive model are shown for these cases in Fig. 6. The two remaining cells each had visible sag back in all traces, and inclusion of the H current in the model was seen to improve the fit to data. For cell 518, the inclusion of the A current in the model provided further improvement, whereas the fits achieved to the data from cell 815 were not improved with the additional current. The comparison of active versus passive model fits for these two cells are shown in Fig. 7.

For both cells 518 and 815, it was determined that the best fits could only be achieved if the voltage dependence of the H current was shifted between 25 and 30 mV in the depolarized direction. The values determined for $G_H$ varied between 19 and 78 $\mu$S/cm$^2$. There was a wider variation in the shifts in the voltage dependence of the A current that were necessary to achieve good fits. The shift required for a good fit to the data for cell 518 was 10 mV in the depolarized direction, for cell 810 no shift was required and for cells 225 and 526 shifts of 10–15 mV in the hyperpolarized direction were required. There was also a fairly wide variation between cells in the value for $G_A$ (148–610 $\mu$S/cm$^2$), although the ranges for the individual cells all varied by $<40\%$. The averages across cells, determined as above, were as follows: $G_H = 50 \pm 40 \mu$S/cm$^2$; $G_A = 380 \pm 200 \mu$S/cm$^2$.

**Contribution of active conductances to resting membrane potential**

Although the H and A currents clearly contributed to the behavior of these cells near the resting membrane potential, it is possible that this was a result of an electrode-induced shunt and that in the cell’s natural resting state those currents would not have been activated. To test this, the resting mem-
brane potential was calculated for each cell as follows: resting membrane potential was calculated with the somatic shunt set to zero, to establish a baseline, and then with the active conductances set to zero to determine their effect. This shift in resting membrane potential was calculated for each of the parameter sets that had a PE within 2% of the minimum PE determined for that cell.

For cell 225, the A current resulted in a 2- to 4-mV hyperpolarization to the rest potential, which varied between −67 and −59 mV. For cell 526, the A current resulted in a 4- to 5-mV hyperpolarization to the rest potential of between −54 and −55 mV. For cell 810, the A current produced a 1- to 5-mV hyperpolarization to the rest potential of between −65 and −76 mV. For cell 815, the H current produced a 16-mV depolarization to the resting membrane potential of −54 mV. Both the H and A current contributed to the rest potential of in cell 518; the A current produced a 9- to 13-mV hyperpolarization and the H current produced a 5-mV depolarization from the resting membrane potential of −54 to −55 mV.

**Discussion**

We have shown that a passive membrane model fails to provide an adequate explanation for the behavior of the LDT neurons close to the resting membrane potential. Even when good fits could be found, often the associated parameters were not physiologically plausible, having extremely high intracellular resistivities or extremely low membrane conductances. A model that included the A current and/or the H current provided better fits with more reasonable parameters. The parameter extraction done using the active conductance model produced values that were generally within the range of that reported for other cells, with the intracellular resistivity ranging no higher than 800 Ω-cm and the membrane conductance between 8 and 89 μS/cm². Because of the addition of those active conductances, voltage responses outside the range used in the fitting also could be evaluated as to how well they were fit by the parameters. This additional constraint led to a much smaller range of effectively indistinguishable parameters in cases where the range determined by the passive model was quite large. This seems a promising method for studying subtheshold cell behavior while minimizing the number of channel blockers introduced into the cellular environment. An interesting property of the active conductances to emerge from the modeling is that the voltage dependence of H-current activation seems to be shifted several mV for intracellular resistivity values for which good fits to data could be found was well outside the ranges reported for any other cells. One cell had a minimum intracellular resistivity of 1,800 Ω-cm and another had a minimum acceptable value of 2,200 Ω-cm. These values would suggest that the behavior of the cells is not sufficiently linear to be described by a passive model.

Although fitting both the long and short pulses simultaneously constrained the parameter values that would provide good fits to some degree, nonuniqueness of parameters still proved to be a serious problem. The ranges of parameters that provided effectively indistinguishable fits were quite large, ranging over as much as six orders of magnitude in the case of membrane conductance. Because of these large ranges, this methodology did not prove to be very useful in extracting membrane parameters.

**Passive model**

One problem with the results of the passive membrane parameter extraction protocol was that the parameters determined in some cases were beyond the bounds of what was physiologically plausible. For some cells, the entire range of intracellular resistivity values for which good fits to data could be found was well outside the ranges reported for any other cells. One cell had a minimum intracellular resistivity of 1,800 Ω-cm and another had a minimum acceptable value of 2,200 Ω-cm. These values would suggest that the behavior of the cells is not sufficiently linear to be described by a passive model.

**Active model**

The parameter extraction done with the active conductances produced values of the capacitance close to the generally accepted value of 1 μF/cm² (Brown et al. 1981; Cole
FIG. 6. Comparison of the active and passive fits for those cells in which the active membrane model did not include the H current. Parameters shown are those that produced the best fit to the long and short pulse responses in a given row. A: for cell 225, both the short pulse passive (A1a) and active (A1b) fits and the long pulse passive (A2a) and active (A2b) fits are similar. This is true of both the traces that were used in the fitting procedure (short pulses and starred long pulses) as well as the additional voltage responses. Parameters are quite similar for the 2 cases.

B: short pulse passive (B1a) and active (B1b) fits and long pulse passive (B2a) and active (B2b) fits are also quite similar for cell 526, for both the traces used in the fitting (short pulses and starred long pulses) and the additional voltage responses. However, the values of \( C_m \) and \( R_i \) are much smaller and closer to typically reported values for the active model. C: while the fits to the long and short pulse responses used in the fitting procedure (*) as well as to the additional short pulse response do not show much visible improvement of the active over the passive model fit for cell 810, there is a dramatic improvement in the active model fit to the additional long pulse responses (C2b) over the passive model fit (C2a). Also, the values of \( R_i \), \( G_m \), and the shunts are all much lower for the active model.

Values for the intracellular resistivity varied from 100 to 800 \( \Omega \)-cm. The upper end of this range is somewhat high relative to the value of 400 \( \Omega \)-cm, the highest intracellular resistivity reported for other cells (Major et al. 1994; Spruston and Johnston 1992; Thurbon et al. 1994; Ulrich et al. 1994). A number of factors may account for this; properties of the cytoplasm and cytoskeletal elements may contribute to high \( R_i \), while errors in this estimate may stem from factors such as noncircular dendritic cross-sections, nonnegligible extracellular resistivity or nonuniformity of resistivity (as discussed in Major et al. 1994; Shelton 1985). Also, there may be an overestimate in \( R_i \) resulting from errors in the recovered morphology due to the resolution constraints...
inherent in viewing the cell with a light microscope (as discussed in O’Boyle et al. 1993, 1996). Although such a high value for $R_i$ would hamper signal propagation in a purely passive dendrite, it may enhance transmission in processes with active conductances. The high $R_i$ would decrease the spread of signal and therefore increase the local response to synaptic input, which could be more effective at activating local voltage-dependent conductances.

The inability of the passive model to fit the data led us to develop a more realistic model for the subthreshold behavior of LDT neurons. Although this introduced more parameters to fit the data, it also opened up a wider variety of data on which to test the model. It appears that the additional information outweighed the addition of more parameters in that the resulting parameter ranges often are decreased in extent.

A dramatic improvement in the fits to the data from cells 518 and 815 was observed when the $V_{1/2}$ of H-current activation was shifted in the depolarized direction. The best fits were for a shift in this value from $-75 \text{ mV}$ to approximately $-45$ to $-50 \text{ mV}$. While the H current has been observed in LDT cells (Leonard and Llinás 1990, 1994; Rainnie et al. 1994), it has not been studied in detail so no experimental data are available to confirm this observation.

A further, preliminary observation was made with respect to the active parameters; the cells appeared to fall into two groups based on the strength of the H current, with only two of the five cells requiring the presence of an H current to adequately model the observed voltage behavior. When the somatic shunts were “removed,” the resting membrane potential for those cells without the strong H current varied from $-59$ to $-76 \text{ mV}$, whereas the resting membrane potential for the two cells that exhibited a strong H current was between $-55$ and $-54 \text{ mV}$. Moreover, simulations indicated that differences in H current strength could alter the integrative properties of these neurons (data not shown). Because all the studied cells were classified as LDT type II neurons on the basis of their physiological properties and no divisions were observed in their cell morphology (Surkis et al. 1996), these findings suggest that LDT type II cells may be composed of more than one physiological subtype. A similar conclusion recently was reached regarding neurons in the rat PPT (Takakusaki et al. 1997). In rat, type II cholinergic PPT neurons were found to fall into two groups (termed short- and long-spike-duration neurons) on the basis of action potential duration and repetitive firing properties. Although this distinction has not been reported for LDT neurons, all of the cells analyzed in our study would fall into the long-spike duration category described in rat PPT. Hence, differences in H-current strength among these cells would represent an additional level of diversity among mesopontine cholinergic neurons. Given the heterogeneity of behavioral-state related firing described for putative cholinergic neurons in the LDT and PPT (see INTRODUCTION), an important future direction will be to relate differences in membrane properties to the observed functional differences among these cells.

APPENDIX

Method for parameter extraction on simulated data using active membrane model

We considered it necessary to test the characteristics of the parameter extraction methodology before using it with an active membrane model. While a similar methodology has been used to extract passive membrane parameters (Major 1992; Major et al. 1994; Thurbon et al. 1994), there is an increase in the number of free parameters to be determined when an active membrane model is used which presents a potential problem with regard to the uniqueness of the extracted parameter sets. Performance of the parameter extraction protocol was tested as follows. The NEURON software package (Hines 1989) was used with the cell geometry from one of the traced LDT cells to simulate the voltage responses...
FIG. 7. Comparison of the active and passive fits for those cells in which the active membrane model included the H current. Parameters shown are those that produced the best fit to the long and short pulse responses in a given row. A: fits to the short pulse response are similar for the active (A1b) and passive cases (A1a) for cell 518, whereas the fits to the long pulse responses are clearly better for the active model (A2b) than for the passive (A2a). This is the case for both the traces used in the fitting procedure (*) and the other voltage traces. Although $C_m$ is quite similar for the 2 cases, both $R_i$ and $G_m$ are approximately twice as large for the passive case, and the shunt values are also much higher for the passive case. B: fits to the short pulse responses for cell 815 are slightly better for the active model (B1b) than for the passive (B1a). Fits to all the long pulse responses, both those used in the fitting (*) and the additional traces, are dramatically better for the active model (B2b) than the passive (B2a). Values for $C_m$, $R_i$, $G_m$, and the shunt values are all lower for the active model.

to both long, low amplitude and short, large amplitude current injections at the soma. These data then were used as the recorded voltage responses to be fit in the parameter extraction protocol, where parameters were extracted for a number of cases where the voltage dependencies of the active conductances or the reversal potentials of any of the conductances were fixed to incorrect values.

Voltage dependence and kinetics of the parameters for the H and A current were the same as those described for the active model in the METHODS. The value for $G_H$ was set at 200 $\mu$S/cm$^2$, which produced a characteristic sag-back in the simulated voltage response to current pulses that was in the range observed in LDT cells in both magnitude and voltage-dependence. The value for $G_A$ also was set at 200 $\mu$S/cm$^2$ to reproduce experimentally observed patterns. The passive parameters used in the simulation were taken as typical values from the literature (Major et al. 1994; Rapp et al. 1994; Spruston and Johnston 1992), as follows

$C_m = 1.0 \mu F/cm^2$

$G_m = 50 \mu S/cm^2$

$R_i = 400 \Omega$-cm

$G_sh = 3.2$ nS.

Residuals were calculated to voltage responses that were used in the fitting. Additionally, the determined membrane parameters were used to calculate voltage responses to current pulse levels.
that were not used in the fitting. The goodness of these fits was assessed by eye.

Simulations of parameter extraction protocol with active membrane model

The method initially was verified by assigning the correct values to each of the fixed parameters and attempting to recover the known parameters; this resulted in recovery of the correct parameters. After this test, errors in the following fixed parameters were considered: reversal potentials for the generalized leak conductance and the somatic shunt conductance, reversal potential and voltage dependence of activation for the H current, and reversal potential and voltage dependence of activation and inactivation for the A current.

When the H-current reversal potential, $E_{\text{H}}$, was fixed at values $\pm 10 \text{mV}$ to the value used in the simulation or the voltage dependence of the activation curve was shifted by $\pm 10 \text{mV}$ in either the hyperpolarizing or the depolarizing direction, excellent fits to the simulated data were found and reasonably good fits were found to the additional voltage traces calculated using the “correct” parameters. Errors in the extracted parameters mainly were confined to errors in $G_{\text{m}}$. While the extracted values of $G_{\text{m}}$ were in error by as much as 102%, errors in $R_{\text{m}}$ were always less than 13% and errors in the remaining parameters were always $<10\%$.

When the A-current reversal potential (assumed to be the potassium reversal potential) was shifted $\pm 5 \text{mV}$ or the voltage dependence of A-current activation was shifted $\pm 5 \text{mV}$, excellent fits were found to both those voltage traces used in the fitting procedure and to the additional calculated voltage responses. The errors in the recovered parameters were concentrated in $G_{\text{m}}$, with errors in other parameters not exceeding 6%. Deviations in the reversal potential resulted in errors of $<15\%$ in $G_{\text{m}}$, whereas the 5-mV shifts in the voltage dependence of activation resulted in errors as large as 144% in $G_{\text{m}}$. When the voltage dependences of A-current activation and inactivation were both shifted $\pm 10 \text{mV}$, thereby shifting the position of the window current relative to the cell’s resting membrane potential, reasonably good fits were achieved to the voltage traces used in the parameter extraction but there were large errors visible in fits to the additional voltage traces. These large visible errors in the fits were accompanied by large errors not only in $G_{\text{m}}$, but in other extracted parameters as well. A 10-mV shift in the hyperpolarizing directions resulted in a 91% error in the recovered membrane capacitance, a 392% error in the recovered intracellular resistivity, and a 51% error in the recovered membrane conductance, along with a 98% error in the recovered value for $G_{\text{m}}$.

A number of errors in the values chosen for the reversal potentials of the generalized leak conductance and/or the somatic shunt conductance were examined. Two simulated data sets were used in this analysis, one with a shunt reversal potential of 0 mV and one with a shunt reversal potential of $-60 \text{mV}$. The two data sets were chosen to simulate both the case of a nonselective shunt, which would reverse at 0 mV, and the case of an electrode-induced shunt, which reverses close to the resting membrane potential, as has been demonstrated more recently in comparisons between whole cell and sharp electrode recordings (Pongracz et al. 1991; Staley et al. 1992). The value for $E_{\text{m}}$ was chosen to be $10 \text{mV}$ to the true value. Parameters were extracted with $E_{\text{m}}$ set to $-30 \text{mV}$ for both the data set calculated with an $E_{\text{m}}$ of 0 mV and the data set calculated with an $E_{\text{m}}$ of $-60 \text{mV}$. Finally, the parameter extraction protocol was run with $E_{\text{m}}$ set at $10 \text{mV}$ hyperpolarized to the true value and $E_{\text{m}}$ set to $-30 \text{mV}$ for both data sets. All of these errors in fixed parameters resulted in excellent fits to both the data used in the fitting and the additional voltage responses with small errors in $R_{\text{m}}$ ($<14\%$) and $C_{\text{m}}$, $G_{\text{m}}$, and $G_{\text{A}}$ ($<8\%$).

Larger errors were found in both $G_{\text{m}}$ and $G_{\text{A}}$ for errors in either $E_{\text{m}}$ or $E_{\text{m}}$. Errors in $G_{\text{m}}$ were as large as 76% for an error in either $E_{\text{m}}$ or $E_{\text{m}}$ and as large as 105% for errors in both $E_{\text{m}}$ and $E_{\text{m}}$. Errors in $G_{\text{A}}$ were as large as 36% for an error in either $E_{\text{m}}$ or $E_{\text{m}}$ and as large as 47% for errors in both $E_{\text{m}}$ and $E_{\text{m}}$. When both $E_{\text{m}}$ and $E_{\text{m}}$ were fixed to incorrect values, the errors from the cases of one parameter being fixed to an incorrect value combined in a roughly linear manner. The larger errors in $G_{\text{m}}$ are less critical since the shunt is assumed to be an artifact of the presence of the electrode in the cell.

Implications of protocol simulations

When the active model parameter extraction protocol was run on voltage traces from simulated current-clamp recordings, the results indicated that the methodology was effective. When incorrect assumptions were made about the values of the fixed parameters, either the errors in both the fits to simulated data and the extracted values for most parameters were small and thus acceptable, or when errors in the extracted parameters were large, the errors in fits to simulated data were also large and thus detectable. These results indicate that errors in fixed parameters for the active conductances should not prevent accurate recovery of the other cable parameters, while recovered values in $G_{\text{m}}$ and $G_{\text{A}}$ can be quite inaccurate. However, results of the actual parameter fitting did indicate that, at least for some cells, sensitivity to these active conductance fixed parameters is such that only a very narrow window of values will result in good fits to recorded data, so the values of $G_{\text{A}}$ and $G_{\text{m}}$ may be more constrained than was indicated in the simulations. Although reliable recovery of the magnitudes of the voltage-dependent conductances cannot be assumed, the reliability of recovery of $R_{\text{m}}$ and $C_{\text{m}}$ is quite encouraging, with the errors in recovery for these simulations being $<15$ and $5\%$, respectively. The errors in $G_{\text{m}}$ were of more concern, ranging as high as 47% in the cases considered. However, errors of this magnitude are within the spread of values seen for the range of acceptable values from the actual parameter extractions. Also, it was seen that the errors in the leak reversal potential and conductance offset each other over the membrane potential range considered to a sufficient degree as to produce negligible errors in the observed fits. It is therefore not unreasonable to imagine that these errors would cancel each other to a sufficient degree throughout the physiologically realistic range of membrane potentials so that the cell behavior would still be modeled accurately.

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