Simulations to Derive Membrane Resistivity in Three Phenotypes of Guinea Pig Sympathetic Postganglionic Neuron

John Jamieson, Hugh D. Boyd, and Elspeth M. McLachlan

Prince of Wales Medical Research Institute, Randwick, New South Wales 2031; and the University of New South Wales, New South Wales 2052, Australia

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Simulations to derive membrane resistivity in three phenotypes of guinea pig sympathetic postganglionic neuron. J Neurophysiol 89: 2430–2440, 2003; 10.1152/jn.01000.2002. The electrotonic behavior of three phenotypes of sympathetic postganglionic neuron has been analyzed to assess whether their distinct cell input capacitances simply reflect differences in morphology. Because the distribution of membrane properties over the soma and dendrites is unknown, compartmental models incorporating cell morphology were used to simulate hyperpolarizing responses to small current steps. Neurons were classified as phasic (Ph), tonic (T), or long-afterhyperpolarizing (LAH) by their discharge pattern to threshold depolarizing current and are modulated by peptidergic transmitters. All three classes of neurons have comparable somatic dimensions. Cell input capacitance, derived from voltage responses to small hyperpolarizing steps from resting potential (Fig. 1C), has previously been found to differ between the classes roughly in proportion to the differences in total estimated surface area of neuronal membrane (Boyd et al. 1996).

The passive electrical properties of multipolar neurons reflect the distribution of ion channel conductances throughout the distributed capacitance provided by the lipid bilayer of the plasma membrane. These properties determine the integrative behavior of neurons during synaptic bombardment (Jack et al. 1975; Rall 1977; Rall et al. 1992; Redman 1976; Segev et al. 1995). To investigate the electrical behavior of central neurons, the morphology of the soma and dendritic tree has been revealed after intracellular dye injection and correlated with detailed analyses of passive voltage and current transients recorded from the soma of the same cell (Major et al. 1994; Stuart and Spruston 1998; Thurbon et al. 1998). Computational models based on such experimental data have been generated that simulate the electrotonic voltage responses (see e.g., Rall et al. 1992; Segev et al. 1995), and more complex models that incorporate voltage-dependent behavior have also been developed (Cook and Johnston 1997; McCormick and Huguenard 1992).

The first electrotonic models were mathematically simplified by reducing the entire dendritic tree to a single “equivalent cylinder” linked to an R-C circuit representing the soma. This is acceptable provided that the diameters of successive levels of dendritic branching follow the “$d^{0.5}$” rule, which describes progressive tapering of the dendritic tree, as for alpha-motoneurons (Clements and Redman 1989), but this rule does not apply to most neurons. The existence of dendrites of unequal electrical lengths places an even greater limitation on the suitability of the single equivalent cylinder model. More recently, compartmental models have been used in which the distribution of ion channel conductances throughout the distributed capacitance provided by the lipid bilayer of the plasma membrane. These properties determine the integrative behavior of neurons during synaptic bombardment (Jack et al. 1975; Rall 1977; Rall et al. 1992; Redman 1976; Segev et al. 1995). To investigate the electrical behavior of central neurons, the morphology of the soma and dendritic tree has been revealed after intracellular dye injection and correlated with detailed analyses of passive voltage and current transients recorded from the soma of the same cell (Major et al. 1994; Stuart and Spruston 1998; Thurbon et al. 1998). Computational models based on such experimental data have been generated that simulate the electrotonic voltage responses (see e.g., Rall et al. 1992; Segev et al. 1995), and more complex models that incorporate voltage-dependent behavior have also been developed (Cook and Johnston 1997; McCormick and Huguenard 1992).

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Manipulating the parameters of these models can predict the

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Address for reprint requests: E. M. McLachlan, Prince of Wales Medical Research Institute, Gate 1, Barker St., Randwick, NSW 2031, Australia (E-mail: e.mclachlan@unsw.edu.au).
FIG. 1. Three classes of sympathetic postganglionic neuron with different electrophysiology and morphology and distinct passive properties. A: records of membrane potential (top) during depolarizing and hyperpolarizing current steps (bottom) showing 3 distinct patterns of action potential discharge. Phasic (Ph) neurons fire in an initial burst, tonic (T) neurons fire throughout the depolarization, and long afterhyperpolarizing (LAH) neurons fire only once due to a prolonged outward current (shown in the top insert). Calibrations apply throughout. B: examples of biocytin filled neurons of each class. Ph neurons (top) have small cell bodies and intermediate dendritic arbors. T neurons (middle) have large cell bodies and the most extensive dendritic arbors, whereas LAH neurons (bottom) have large cell bodies with the least extensive dendrites. To the right are average representations of the soma (black ellipse) and dendrogram of each neuron class. C: examples of normalized voltage transients in response to current steps (timing shown below middle trace). These responses were averaged for 5 neurons in each class, a subset of the 58 cells included in the present analysis. To the right, the voltage transients are plotted semilogarithmically with lines of best fit superimposed. (Note that A and B are reproduced from Boyd et al. 1996 and the traces in C are illustrative only).
The means $\pm$ SE of these data have been used as the "targets" in the model analysis. Regression lines fitted through the points (and the origin) have $r^2 = 0.84$ (tonic), 0.88 (phasic), and 0.89 (LAH).

possible distributions of channels underlying a neuron’s behavior. In electrotone models, only three properties can be varied: specific membrane resistivity ($R_m$), specific membrane capacitance ($C_m$), and specific resistivity of the axoplasm ($R_p$). The effects on model behavior are therefore determined primarily by the structural components of the model. It has usually been assumed that the distribution of membrane conductance is uniform, although this is clearly not always the case (Campbell and Rose 1997). Such a model applied to sympathetic neurons should predict the input capacitance from the morphological characteristics provided that membrane resistivity is the same for neurons of all classes.

A common finding in such studies is that the cell input capacitance, derived by dividing the cell input time constant, $\tau_0$, by the cell input resistance, $R_N$, is larger than that calculated by multiplying the estimated surface area of the filled neuron by $C_m$, assuming the widely accepted value of 1 $\mu$F cm$^{-2}$ (Jack et al. 1975). In studies where $C_m$ has been derived rather than assumed, the common finding has been that $C_m \approx 1$ $\mu$F cm$^{-2}$ (Lux et al. 1970; Nitzan et al. 1990; Rapp et al. 1994; Thurbon et al. 1998). Several possible explanations have been put forward. One is that $C_m$ is actually higher than 1 $\mu$F cm$^{-2}$ (Thurbon et al. 1998). Another explanation is that there may be a somatic shunt conductance, i.e., the somatic membrane is much leakier than the dendritic membrane, either because of a higher density of leak channels in the soma than in the dendrites or as an artifact of microelectrode impalement (Barrett and Crill 1974). It is also possible that membrane surface area (SA) has been underestimated, and studies that attempt to account for this trend to agree that $C_m = 1$ $\mu$F cm$^{-2}$ (Gentet et al. 1999; Stuart and Spruston 1998).

Here we have compared the measured passive electrical properties of each class of sympathetic neuron with those calculated from cell dimensions, initially assuming $C_m = 1$ $\mu$F cm$^{-2}$. We used a compartmental model (Hines and Carnevale 1997) to predict the voltage transients that would be recorded in the soma. By modifying the parameters of the model in feasible ways, the electrotone voltage transients recorded experimentally were mimicked. The resting membrane was assumed to be passive and of uniform resistivity, i.e., its impedance was linear without voltage- or time-dependent conductances. The adequacy of some assumptions has been assessed, and the effect of a possible somatic shunt conductance has been investigated. The results imply that the values of resting membrane conductance differ between the three classes of sympathetic neuron.

**METHODS**

**Electrophysiology**

Details of the electrophysiology experiments have previously been described (Boyd et al. 1996). Sympathetic ganglia were dissected from young adult guinea pigs perfused with physiological saline under deep urethane anesthesia (1–1.5 g/kg ip). Neurons were impaled in vitro at 35°C with high-resistance microelectrodes (80–150 MΩ) containing 2% biocytin and studied with single electrode current and voltage-clamp techniques. Biocytin filled the cells during current switching. Neurons were classified as T, Ph, or LAH by their discharge characteristics during graded depolarizing current steps (Fig. 1A) and by the presence or absence of certain voltage- and Ca$^{2+}$-dependent conductances (Boyd et al. 1996; Keast et al. 1993; McLachlan and Meckler 1989).

Passive electrical properties were determined from recordings in current clamp of small amplitude (<10 mV) responses that avoided activating voltage-dependent conductances in these neurons (Cassell and McLachlan 1987; Cassell et al. 1986). Hyperpolarizing current steps were used rather than brief current pulses (Jack et al. 1975) because of current-passing limitations of high-resistance electrodes. Voltage and current were filtered (1–3 kHz) and digitized at $\approx$1 kHz.

The cell input time constant, $\tau_0$, was derived by fitting a single exponential to the averaged response between 20 and 80% of the membrane potential; $R_{MP}$, resting membrane potential; $+\text{ , } -\text{ , values are means and ranges extending } \pm\text{SE.}$

**TABLE 1A.** Average passive properties of each class of sympathetic neuron

<table>
<thead>
<tr>
<th>$R_N$ (MΩ)</th>
<th>$\tau_0$ (ms)</th>
<th>$R_{MP}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic</td>
<td>122 138 154</td>
<td>30 34 38</td>
</tr>
<tr>
<td>Phasic</td>
<td>141 157 173</td>
<td>21 24 27</td>
</tr>
<tr>
<td>LAH</td>
<td>85 101 117</td>
<td>11 13 15</td>
</tr>
</tbody>
</table>

Data from 22 tonic, 18 phasic, and 18 LAH neurons are from Boyd et al. (1996). $R_N$, cell input resistance; $\tau_0$, cell input time constant; $R_{MP}$, resting membrane potential; +, −, values are means and ranges extending $\pm$SE.
TABLE 1B. Average morphology of each class of sympathetic neuron

<table>
<thead>
<tr>
<th>Cell Body Axes</th>
<th>Axon</th>
<th>Dendrites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor (μm)</td>
<td>Major (μm)</td>
<td>Diameter (μm)</td>
</tr>
<tr>
<td>Tonic</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td>Phasic</td>
<td>25</td>
<td>39</td>
</tr>
<tr>
<td>LAH</td>
<td>29</td>
<td>44</td>
</tr>
</tbody>
</table>

Values are from Boyd et al. (1996). Figure 1B shows scaled dendrograms of the averaged models used.

Modeling

To focus on class differences, the aim was to match the \( R_m \) and \( \tau_m \) simulated in the models with the average electrical properties and morphologies derived experimentally (Boyd et al. 1996) rather than analyzing data or fitting voltage transients from single neurons.

The average morphologies described above (see also Fig. 1B) were incorporated into compartmental models using the program NEURON (Hines and Moore 1999), which uses cylindrical representations of the soma and processes. The cell body was a cylinder with equal diameter and length, set to the average of the measured cell body major and minor axes (Table 1B), so it had the same SA as a sphere with this average diameter. The axon was given constant diameter and length equal to the average measured values for each class (Table 1B), and the dendrograms in Fig. 1B were used with the average branching patterns for each class fully implemented (see Boyd et al. 1996). The dendrites of each class had constant diameters that were equal to the measured average for that class (Table 1B). As a compromise between accuracy and time for each simulation (Hines and Carnevale 1997), each morphological segment was subdivided into only five variable length compartments using the NEURON software (Hines and Moore 1999). The inaccuracy associated with representing long segments of axon as a single compartment was estimated by performing some simulations using 1-μm-long compartments. The differences were less than the SE of the experimental passive properties (Table 1A) and have been ignored.

Simulations were performed with a sampling frequency of 1 kHz, which matches the slowest experimental sampling. The models were entirely passive, with no voltage- or time-dependent impedances. The model parameters were assumed to be uniform throughout the cell, except where specified.

The models had five parameters: 1) \( C_m \), the specific membrane capacitance, taken as 1 μFcm\(^{-2}\); 2) \( R_m \), the cytoplasmic resistivity, the same for all three classes with values between 70 and 2,500 Ωcm tested (Barrett and Crill 1974; Cole 1968; Stuart and Spruston 1998); 2) \( R_m \), the specific membrane resistance, uniform over the entire cell, except in simulations with a somatic shunt, and allowed to vary between classes in steps of 1 kΩcm\(^{-2}\); 4) SA corrections implemented by scaling diameter or SA directly (see next paragraph); and 5) \( \delta_{shunt} \), a somatic shunt conductance.

The constant diameter cylinder representation fails to allow for surface irregularities and for the possibility of histological shrinkage. One correction (A) was implemented by equally scaling cell body, axon, and dendrite diameters. For example, multiplying all cylinder diameters by 1.25 scaled the modeled neuron SA by 1.25, a 25% SA increase. A second method of SA correction (B) did not change diameters but artificially scaled the SA directly (as in Stuart and Spruston 1998) by increasing \( C_m \) and decreasing \( R_m \) by a common factor, thereby maintaining \( \tau_m \), the membrane time constant.

\( \delta_{shunt} \) accounts for a possibly leaky cell body (Clements and Redman 1989; Pongracz et al. 1991; Staley et al. 1992) and was modeled by reducing the somatic \( R_m \) uniformly. Such a shunt might, in reality, consist of a transmembrane conductance and/or a leak around the microelectrode.

Voltage responses at the soma to hyperpolarizing current steps of 250 ms duration, as in the electrophysiology measurements (Fig. 1C), were simulated. Values of \( \tau_m \) and \( R_m \) were derived for fitting a single exponential to the first 100 ms of this response using the praxis curve-fitting module within NEURON. Single exponentials provided very good fits to the modeled responses. It should be noted that, in neurons with branched processes, \( \tau_m \) is not necessarily the same as \( \tau_m \) (Humes et al. 1992).

The aim of the simulations was to find model parameter combinations and ranges that gave voltage responses with \( R_m \) and \( \tau_m \) that matched the corresponding average experimental values, \( R_m \) and \( \tau_m \), for each neuron class. The difference between modeled and experimental passive properties was standardized to remove bias of \( R_m \) over \( \tau_m \) because of larger numerical values. The values that provided the closest match were those that minimized the expression \( \sqrt{[(R_m - R_{exp})/R_{exp}]^2 + [(\tau_m - \tau_{exp})/\tau_{exp}]^2]} \), where \( R_{exp} \) and \( \tau_{exp} \) are the SE of \( R_m \) and \( \tau_m \), respectively.

To investigate the impact of the correlation between \( R_m \) and \( \tau_m \) some simulations were performed using \( R_m \) and cell input capacitance, defined as \( C_m = \tau_m/R_m \), as the model outputs. The results were more or less indistinguishable from those obtained for \( R_m \) and \( \tau_m \) and lay within much less than 1 SE of the experimental values (Table 1A).

RESULTS

Models with uniform \( R_m \) and uncorrected morphology

Initial simulations were performed with uniform \( R_m \) and \( R_f \) of 100 Ωcm (Barrett and Crill 1974; Burke et al. 1994; Cole 1968; Stuart and Spruston 1998). The \( R_m \) and \( C_m \) values obtained were smallest for the LAH class (12 kΩcm\(^2\) and 1.25 μFcm\(^{-2}\)), intermediate for the Ph class (18 kΩcm\(^2\) and 1.5 μFcm\(^{-2}\)), and largest for the T class (23 kΩcm\(^2\) and 1.75 μFcm\(^{-2}\)). The ranges of \( R_m \) and \( C_m \) that gave modeled \( R_f \) and \( \tau_m \) within ±1 SE of the mean experimental values were also determined (Table 2). However, all neurons are expected to have the same \( C_m \). Using a median \( C_m \) value of 1.5 μFcm\(^{-2}\), the simulated values matched the mean experimental \( R_f \) and \( \tau_m \) for all classes within ±1 SE, with little change in \( R_m \), an exact match was only possible for the Ph class.

The possibility of \( C_m \) being as high as 2.5 μFcm\(^{-2}\) was explored (Thurbon et al. 1998). The models could be solved

<table>
<thead>
<tr>
<th>( R_m (\text{kΩcm}^2) )</th>
<th>( C_m (\text{μFcm}^{-2}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic</td>
<td>20</td>
</tr>
<tr>
<td>Phasic</td>
<td>16</td>
</tr>
<tr>
<td>LAH</td>
<td>10</td>
</tr>
</tbody>
</table>

\( R_m \), specific membrane resistivity; \( C_m \), specific membrane capacitance; no surface area correction; +, −, values derived to match the mean passive properties ±SE.
histological shrinkage was therefore considered unnecessary. To chemical staining of intracellular biocytin was undertaken directly in these preparations. However, it is likely to be small for the T or Ph classes when

\[ C_m > 1.5 \mu F/cm^2 \]

by increasing \( R_i \) (Table 3; Fig. 3A). For example, the Ph model could be solved with \( C_m = 1.75 \mu F/cm^2 \) and an acceptable \( R_i \) of 200 \( \Omega \)cm (Rall et al. 1992) (Table 3). For \( C_m = 2.5 \mu F/cm^2 \), the Ph and T responses could be matched using a biologically extreme \( R_i \) of 1,000 \( \Omega \)cm (Clements and Redman 1989; Rall et al. 1992), but the LAH responses required an improbable \( R_i \) of 2,500 \( \Omega \)cm. \( C_m \) in these neurons must be \(<2 \mu F/cm^2 \), purely on the basis of unrealistically high \( R_i \).

There is mounting evidence that \( C_m = 1 \mu F/cm^2 \) for almost all cells (Gentet et al. 1999; Okamoto et al. 1977; Sukhorukov et al. 1993). Using \( C_m = 1 \mu F/cm^2 \), the LAH response could not be matched exactly, but it could be matched within \( \pm 1 \) SE when \( R_i = 70–100 \Omega \)cm. However, a match was not possible for the T or Ph classes when \( C_m < 1.25 \mu F/cm^2 \), except using unrealistically low \( R_i \) (<20 \( \Omega \)cm). This conflicts with the idea that \( C_m = 1 \mu F/cm^2 \) in all neurons. It can be concluded that a systematic error exists that is greater for the T and Ph than for the LAH classes.

Surface area correction is justified

A likely source of systematic error is SA underestimation. Two possible sources of SA underestimation are histological specimen shrinkage and unmapped surface convolutions.

HISTOLOGICAL SHRINKAGE Tissue shrinkage was primarily in the \( z \) axis (Boyd et al. 1996). Cell shrinkage was not measured directly in these preparations. However, it is likely to be small (<5%) and independent of tissue shrinkage, because the histochemical staining of intracellular biocytin was undertaken prior to dehydration (Grace and Llinas 1985). A correction for histological shrinkage was therefore considered unnecessary.

UNDETECTED/IGNORED SURFACE AREA Sympathetic neurons deviate from the computer model comprised of constant diameter cylinder segments in several ways. To varying extents, neurons of all three classes have irregular somatic surfaces and several types of dendritic irregularity, including varicosities, loops, and fine branches (Boyd et al. 1996). As indicated in a summary of the fine surface morphology of these neurons (Table 4), a correction for ignored SA is likely to be necessary. Further, the different classes probably require different corrections. On average, T neurons had the most irregularities and surface convolution, and LAH neurons had the least (Table 4). Determination of the complete extent of surface membrane invaginations would have required complete reconstructions of individual neurons using the electron microscope, which was beyond the scope of this study.

### Table 3. Limits to permissible combinations of \( C_m \) and \( R_i \)

<table>
<thead>
<tr>
<th>( R_i (\Omega )cm)</th>
<th>LAH</th>
<th>Phasic</th>
<th>Tonic</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>1.25</td>
<td>1.50</td>
<td>1.75</td>
</tr>
<tr>
<td>100</td>
<td>1.25</td>
<td>1.50</td>
<td>1.75</td>
</tr>
<tr>
<td>200</td>
<td>1.50</td>
<td>1.75</td>
<td>2.00</td>
</tr>
<tr>
<td>1,000</td>
<td>2.00</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>2,500</td>
<td>2.50</td>
<td>&gt;2.50</td>
<td>&gt;2.50</td>
</tr>
</tbody>
</table>

\( R_i \), specific resistivity of axoplasm. Values correspond to models without surface area correction. The values for LAH provide the limit for the other two classes, if it is assumed that \( R_i \) and \( C_m \) are the same in all classes (see Fig. 3A).

with \( C_m > 1.5 \mu F/cm^2 \) by increasing \( R_i \) (Table 3; Fig. 3A). For example, the Ph model could be solved with \( C_m = 1.75 \mu F/cm^2 \) and an acceptable \( R_i \) of 200 \( \Omega \)cm (Rall et al. 1992) (Table 3). For \( C_m = 2.5 \mu F/cm^2 \), the Ph and T responses could be matched using a biologically extreme \( R_i \) of 1,000 \( \Omega \)cm (Clements and Redman 1989; Rall et al. 1992), but the LAH responses required an improbable \( R_i \) of 2,500 \( \Omega \)cm. \( C_m \) in these neurons must be \(<2 \mu F/cm^2 \), purely on the basis of unrealistically high \( R_i \).

When \( R_i = 100 \Omega \)cm: combinations of \( C_m \) and SA correction factor (correction factor). The lines in this and subsequent figures interpolate between simulated data points for each class. A: combinations of \( C_m \) and \( R_i \) that provided solutions to the uncorrected models with uniform \( R_m \). Only the LAH curve is shown because this limit applies to all 3 classes (see Table 3). Hatching illustrates the combinations of \( C_m \) and \( R_i \) that do not provide solutions for any model, and the arrows indicate the combinations that can theoretically match the experimental data for all classes when a SA correction, as in B and C, is applied. B: combinations of \( C_m \) and SA correction factor (correction factor A) that provided solutions to the uncorrected models with uniform \( R_m \) and constant \( R_i \). Curve illustrates the case for \( R_i = 100 \Omega \)cm. Note that there are solutions for all 3 classes only when \( C_m < 1.25 \mu F/cm^2 \). C: combinations of \( R_i \) and SA correction factor (correction A) that provided solutions to the uncorrected models with uniform \( R_m \) and constant \( C_m \). Curve illustrates the case for \( C_m = 1 \mu F/cm^2 \). Error bars in this and subsequent figures represent the values derived to match the mean passive properties \( \pm \) SE (see Fig. 4).
TABLE 4. Surface features of neuron classes that contribute to underestimation of surface area

<table>
<thead>
<tr>
<th>Tonic</th>
<th>Phasic</th>
<th>LAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendrites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of neurons with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mainly smooth dendrites</td>
<td>“many”</td>
<td>73%</td>
</tr>
<tr>
<td>Some varicose dendrites</td>
<td>52%</td>
<td>50%</td>
</tr>
<tr>
<td>Some lumpy or bulbous dendrites</td>
<td>15%</td>
<td>11%</td>
</tr>
<tr>
<td>Some irregular dendrites with fine branches</td>
<td>60%</td>
<td>32%</td>
</tr>
<tr>
<td>Soma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soma outline</td>
<td>Notably irregular</td>
<td>Usually oval</td>
</tr>
<tr>
<td>Axes (2a, 2b) (μm)</td>
<td>180 ± 56</td>
<td>130 ± 36</td>
</tr>
<tr>
<td>( P_i = \pi(a + b) (\mu m) )</td>
<td>126</td>
<td>101</td>
</tr>
<tr>
<td>Mean ( P/P_i )</td>
<td>1.43</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Data in all but the last two rows are from Boyd et al. (1996). \( P_i \), perimeter of the soma projection; \( P \), diameter of circle with diameter equal to mean of axes 2a and 2b; \( P/P_i \), measure of the extent of convolution of the surface of the soma; \( P_i \), perimeter of an ellipse (Ramanujan 1962) with major and minor axes 2a and 2b, by \( \approx \leq 2 \, \mu m \).

Models with uniform \( R_m \) and surface area correction

To account for SA underestimation, simulations were repeated using a variable SA correction applied \( A \) by increasing the diameters of all morphological segments and \( B \) by increasing SA directly (see METHODS).

Increasing diameter of each compartment

INTERACTION BETWEEN SURFACE AREA CORRECTION \( A \) AND \( C_m \)

Increasing SA decreased the required values of \( C_m \) (Fig. 3B). With \( R_i = 100 \, \Omega cm \), solutions were obtained using \( C_m \) values that decreased by about 0.05–0.2 \( \mu Fcm^{-2} \) for each 10% of SA increase. The T class required the most correction and the LAH class required the least. The LAH class could not be solved with \( C_m > 1.25 \, \mu Fcm^{-2} \) because this would require a negative correction. For larger \( R_i \), the effect on \( C_m \) was more pronounced.

By increasing the modeled SA by different amounts (in steps of 5%) for each class, solutions could be found with \( R_i = 100 \, \Omega cm \) and \( C_m = 1 \, \mu Fcm^{-2} \) (Fig. 4). \( R_N \) and \( \tau_0 \) could be matched to within ±1 SE of the mean observed \( R_N \) and \( \tau_0 \) by applying SA corrections of +25 ± 15%, +45 ± 10%, and +55 ± 10% for the LAH, Ph, and T classes, respectively. An intermediate SA increase of 45% could solve the models for all three classes, using these values of \( C_m \) and \( R_i \), although this was not an exact solution for the LAH and T classes. However, an equal SA increase for all classes would not account for the differences in surface irregularity (Table 4). The models were consistently solved for all combinations of \( R_i \) and \( C_m \) by applying successively greater (and unequal) SA corrections to the LAH, Ph, and T models (Table 5).

INTERACTION BETWEEN SURFACE AREA CORRECTION \( A \) AND \( R_i \)

Increasing SA with \( C_m \) fixed increased the required values of \( R_i \) (Fig. 3C), as was the case when \( C_m \) was increased when there was no SA correction (Table 3). With \( C_m = 1 \, \mu Fcm^{-2} \), a 10-fold increase in \( R_i \) (from 100 to 1,000 \( \Omega cm \)) corresponded to an increase in the SA correction of about 30% (Fig. 3C). With \( C_m < 1 \, \mu Fcm^{-2} \), this relationship was less sensitive to \( R_i \), and for \( C_m > 1 \, \mu Fcm^{-2} \), it was more sensitive. However, for all \( C_m \), changing \( R_i \) over a wide range had relatively little effect on the SA correction (see Fig. 3C). These models tolerate almost any value of \( R_i \), \( R_i \) is limited by what is biologically realistic rather than any constraint of the modeling.

UPPER LIMITS OF SURFACE AREA CORRECTION \( A \)

The solutions for \( C_m = 1 \, \mu Fcm^{-2} \) and \( R_i = 100 \, \Omega cm \) already described (Table 5) suggest that 65–80% [= (1 + SA correction)\(^{-1}\)] of

![FIG. 4. Matching the passive electrical properties of compartmental models of each neuron class, with experimental \( R_N \) and \( \tau_0 \), by varying \( R_m \) and increasing SA. Hatched boxes are target regions for each model, centered on the average experimental \( R_N \) and \( \tau_0 \) and extending from the center by ± experimental SE for each neuron class. \( C_m = 1 \, \mu Fcm^{-2} \), \( R_i = 100 \, \Omega cm \), and uniform \( R_m \). Optimal \( R_m \) and SA corrections are derived from those simulation points nearest the target region centers, and upper and lower limits are derived from the simulation points nearest the target region edges (see Table 5). Lines link simulation points derived using the same \( R_m \) but different SA corrections. \( R_m \) is distinct, and SA corrections differ for each neuron class.](http://jn.physiology.org/)

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the neuron SA is accounted for by an uncorrected cylindrical representation. However, larger SA corrections could be accommodated by a corresponding large increase in $R_i$ and/or reduction in $C_m$. The lowest realistic $C_m$ value (0.75 $\mu$F/cm$^2$, e.g., Almers 1978) and highest imaginable $R_i$ value (1,000 $\Omega$cm) were used to calculate an upper limit to the SA correction (Fig. 3, B and C). With these values of $C_m$ and $R_i$, the SA corrections were 100% (LAH), 120% (Ph), and 150% (T). In other words, in the most extreme case, the cylindrical representation accounted for only 40–50% of the neuron SA.

**Direct scaling of SA**

The axial resistance of a cylindrical cable with diameter, $d$, is proportional to $R_i/d^2$, so it is possible that some of the modeled insensitivity to high $R_i$ values using correction method A (Figs. 3C and 5B) is a consequence of the increase in axon and dendrite diameters. The second SA correction method was implemented by scaling the SA independently of diameter.

Simulations for all three classes using the preferred $R_i$ and $C_m$ values: $R_i = 100$ $\Omega$cm and $C_m = 1$ $\mu$F/cm$^2$ yielded values of $R_m$ for the three classes that were within 1 k$\Omega$cm$^2$ of the values obtained using correction A. However, about 10% greater SA corrections were necessary with correction B. The T model again required the largest correction, and the LAH model the least, as for correction A.

Thus the two methods of SA correction gave basically the same results, suggesting that increasing the diameter (correction A) may only be inappropriate if $R_i$ is unrealistically high. Method A is also likely to be unsuitable for estimating electrotonic attenuation along the dendrites (Rall et al. 1992), because of underestimation of axial resistance.

**Differences in $R_m$ between neuron classes**

As already stated, $R_m$ values and corresponding $R_m$ ranges were derived for $R_i = 100$ $\Omega$cm and $C_m = 1$ $\mu$F/cm$^2$ by applying SA corrections (Fig. 4). The optimal $R_m$ values were 40, 27, and 15 k$\Omega$cm$^2$ for T, Ph, and LAH classes, respectively. These were obtained using SA corrections (A) of 55, 45, and 25%, respectively (cf. Table 5 and Table 4). The $R_m$ for each class was different, and the $R_m$ ranges were distinct.

**Effect of varying $C_m$ and $R_i$ on derived $R_m$**

The effects on $R_m$ of varying $C_m$ and $R_i$ from 1 $\mu$F/cm$^2$ and 100 $\Omega$cm, respectively, were investigated to make comparisons with other studies. The derived $R_m$ decreased as $C_m$ increased (Fig. 5A). In contrast, $R_m$ derived for large values of $R_i$ (<2,500 $\Omega$cm), differed little from the values derived at lower $R_i$ (Fig. 5B). Even though the predicted $R_m$ changed when $C_m$ was varied, the ranges of $R_m$ for each class remained distinct for all values of $C_m$ (0.75–2.5 $\mu$F/cm$^2$) and $R_i$ (70–2,500 $\Omega$cm), and the relative $R_m$ differences between classes were preserved (Tonic $R_m >$ Phasic $R_m >$ LAH $R_m$).

**Models with a somatic shunt conductance**

The models with uncorrected morphology could not be solved with $C_m$ approximately 1 $\mu$F/cm$^2$. SA underestimation successfully accounted for the discrepancy, but an alternative or contributing error may have resulted from a somatic shunt conductance, $g_{\text{shunt}}$. A somatic shunt conductance, whether intrinsic (due to higher conductance of the soma membrane) or artifactual (introduced by the microelectrode), would reduce the measured $R_N$ and lead to a lower $R_m$ estimate. A high value of $C_m$ (as determined in many studies) would then be required to match the measured $\tau_i$.

**Effects of $g_{\text{shunt}}$ on $C_m$ and $R_i$**

$g_{\text{shunt}}$ affected the models with uncorrected morphology in a complex manner. The relationship between $C_m$ and $R_i$ (Fig. 3A) became skewed to lower $C_m$ but never $<1.5$ $\mu$F/cm$^2$, and only for $R_i \geq 200$ $\Omega$cm and $g_{\text{shunt}} > 3$ nS. Otherwise, the only effect of $g_{\text{shunt}}$ was to increase $R_m$ (see next paragraph). Models with $g_{\text{shunt}}$ could not be solved at all for $C_m = 1$ $\mu$F/cm$^2$ unless a SA correction was also applied.

In the models with SA correction, as $g_{\text{shunt}}$ increased, the same or less SA correction was required. This effect was graded with Ph $>$ T $>$ LAH. When $C_m = 1$ $\mu$F/cm$^2$ and $R_i = 100$ $\Omega$cm, the Ph model required 45% SA increase (type A) if...
there was no shunt, but with \( g_{\text{shunt}} = 4 \text{nS} \), it required only a 35% SA increase. Under these conditions, the SA corrections derived for the T and LAH models were the same with \( g_{\text{shunt}} = 4 \text{nS} \) as those derived for no shunt. Thus, the presence of a somatic shunt conductance, whether real or artifactual, could not account for the \( C_m = 1.5 \mu \text{F/cm}^2 \) derived with no SA correction. A large shunt (\( g_{\text{shunt}} \) approximately 4 nS) did, however, allow some models to be solved for \( C_m = 1 \mu \text{F/cm}^2 \) with about 10% less SA correction than would otherwise be the case.

**EFFECT OF \( g_{\text{shunt}} \) ON \( R_m \).** The main effect of a shunt was to increase the \( R_m \) required to solve the models (Fig. 6). \( R_m \) increased dramatically as \( g_{\text{shunt}} \) was increased toward the mean observed input conductance for each class \( [G_N = 1/(\text{average } R_N) = 7 \text{nS (T)}, 6 \text{nS (Ph)}, \text{and } 10 \text{nS (LAH)}] \). For the models with corrected SA, the \( R_m \) ranges for each class were distinct for \( g_{\text{shunt}} \leq 1 \text{nS} \), but for \( g_{\text{shunt}} > 3 \text{nS} \), the \( R_m \) ranges for the Ph and T classes overlapped. With \( g_{\text{shunt}} > 5 \text{nS} \) and either or both of \( R_i > 100 \Omega \text{cm} \) and \( C_m > 1 \mu \text{F/cm}^2 \), the \( R_m \) derived from the Ph class exceeded that from the T class. However, the range of \( R_m \) for the LAH class remained distinct regardless of the shunt size. \( R_m \) could be the same for all classes only if \( g_{\text{shunt}} < 1 \text{nS} \) for the T neurons, \( g_{\text{shunt}} = 1-2 \text{nS} \) for the Ph neurons, and \( g_{\text{shunt}} > 4 \text{nS} \) for the LAH neurons.

**DISCUSSION**

This study has shown that voltage transients in sympathetic neurons can be modeled using commonly accepted biophysical parameters, resulting in discrete ranges of specific membrane resistance \( (R_m) \) for each of three phenotypic classes (T, Ph, and LAH). The derived values of \( R_m \) were only about 15% higher in each case than values derived assuming that the cells were isopotential. This implies that the neurons are electrotomically compact. It was also concluded that a large proportion of the total SA of these neurons exists as fine surface features.

Measured values of \( R_m \) range from 1 k\( \Omega \text{cm}^2 \) in squid giant axon (Cole and Hodgkin 1939) to 22 and 9 k\( \Omega \text{cm}^2 \) in patches of somatic membrane from brain stem motoneurons of neonatal and juvenile rats, respectively (Singer et al. 1998). Calculations based on simulations similar to the present ones have given values ranging from 0.1 k\( \Omega \text{cm}^2 \) in cat spinal motoneuron somata (Clements and Redman 1989) to 200 k\( \Omega \text{cm}^2 \) in hippocampal pyramidal neurons (Major et al. 1994). Even the most extreme values achieved in the present study were well within this range.

**Differences in \( R_m \) between neuron classes**

The difference in \( R_m \) between the classes was robust and could not be invalid by varying uniform model parameters. The differences cannot be ascribed to morphological differences as these were the basis of the models. Although sympathetic neurons are all derived from the same kind of neuroblast (Leblanc and Bronner-Fraser 1992), the conclusion is that the complement and number of channels open at RMP constitute another phenotypic difference between the three classes of sympathetic neuron defined on the basis of their discharge characteristics.

It is known that certain types of channel active at resting potential are expressed differentially across the three classes of neuron.

1) In LAH neurons, a \( \text{Ca}^{2+} \)-dependent \( \text{K}^+ \) conductance \( (g_{\text{KCa}^2}) \) that is responsible for the long afterhyperpolarization is active at rest (Davies et al. 1999); this conductance is similar to the “K-creep” current that accounts for 10–20% of the resting conductance in some enteric neurons (North and Tokimasa 1987). Consistent with this, nifedipine block of L-type \( \text{Ca}^{2+} \) channels that virtually abolishes the slow afterhyperpolarization affects passive properties only in LAH neurons, depolarizing them slightly and reducing resting conductance by approximately 15% (Davies et al. 1999).

2) In T neurons, the voltage characteristics of inactivation of A-type \( \text{K}^+ \) channels are such that a proportion of these channels are open at RMP (Cassell et al. 1986). Thus blockade of these channels with catechol depolarizes T neurons, reducing their resting conductance and increasing the input time constant (Inokuchi et al. 1997). Neither catechol nor block of \( \text{Ca}^{2+} \) entry have significant effects on the resting conductance of Ph neurons (Davies et al. 1999; Inokuchi et al. 1997).

3) Fewer than 10% of guinea pig sympathetic neurons exhibit time-dependent rectification \( (I_q) \) (Inokuchi et al. 1997), and the time course and amplitude of the afterhyperpolarization in any cell class are not affected by the addition of 2 mM Cs\( ^+ \) to block \( I_q \) (Davies et al. 1999).

Most of these channels will not be affected by the small amplitude hyperpolarizations of the soma used to determine \( R_m \). For example, no more A channels would have been opened than were already open at RMP. Thus it is clear that distinct populations of active channels may contribute to the resting membrane characteristics in each class of sympathetic neuron.
It must be kept in mind that the distribution of these channels is probably not uniform over the somatic and dendritic membrane. For example, the voltage-dependent Ca\(^{2+}\) channels activated during the action potential in rat sympathetic neurons have high thresholds and are located at a site electrically distant from the soma (Hirst and McLachlan 1986), and this may be reflected in the location of Ca\(^{2+}\)-activated K\(^+\) channels. In hippocampal neurons, Ca\(^{2+}\)-activated K\(^+\) channels have been localized to the proximal dendrites (Poolos and Johnston 1999). At this stage, the precise location of the various voltage- and Ca\(^{2+}\)-activated channels in each class of sympathetic neuron is not yet known.

Although it was assumed here, for simplicity in the modeling, that \(R_m\) was spatially uniform over the entire dendritic tree, there is evidence that this is not the case in sympathetic neurons. Leaky distal dendrites were identified in rat paravertebral neurons by the disproportionately larger effect of pharmacological K\(^+\) channel blockade on \(R_N\) than on \(\tau_0\) (Redman et al. 1987). A similar result was obtained in T neurons following blockade of A-type K\(^+\) channels, in that \(R_N\) increased by 30%, whereas \(\tau_0\) increased by only 23% (Inokuchi et al. 1997). This is consistent with the location of these channels in the dendritic membrane, as has been shown for hippocampal neurons (Magee et al. 1998). For neurons, such as sympathetic neurons, with voltage transients that relax with a single relatively large time constant (Fig. 1C) and with distal dendritic membrane that is "leakier" than the proximal dendritic segments, it has been calculated (London et al. 1999) that the total resting conductance is higher than that expected if \(R_m\) is uniform. In other words, there are likely to be more ion channels open at RMP, particularly for T neurons, than suggested by the results of the uniform \(R_m\) modeled in this study. However, removal of this nonuniformity by blockade of open A channels left T neurons with even lower conductance than the other classes of sympathetic neuron (see 2 above), indicating that the presence of active channels in the resting membrane cannot account for the differences in \(R_m\) between classes determined here.

Surface area estimates

Compartmental electrical models incorporating simple cylindrical segments based on gross morphological measurements were unable to mimic the electrical properties of the cells using reasonable values for \(C_m\) and \(R_i\), unless surface irregularities were also accounted for. One correction (A) involved increasing SA by scaling all segment diameters by a variable but uniform amount. The corrections applied differed between classes in accord with the observed details of fine surface morphology, including infolding of the soma surface, dendritic varicosities, short fine processes, and lumpy or bulbous dendrites (Boyd et al. 1996; Gibbins et al. 1998; Kawai et al. 1993).

SAs recently estimated from three-dimensional confocal imaging of sympathetic neurons were much larger than the values estimated here (Anderson et al. 2001; Ermilov et al. 2000). This might have been expected from the higher resolution of the imaging technique. However, because of the limitations of the light microscope, even this technique is unlikely to detect fine surface irregularities at the sub-micron scale. In fact, the somatic SAs and estimates of the dendritic lengths derived in these and other studies (Jobling and Gibbins 1999; Miller et al. 1996) match ours very well. The major difference is in estimates of dendritic SA. It is known that SA estimates based on reconstruction of digital images are prone to errors of bias, particularly for single projection images of nonspherical objects and for images of nonuniform intensity (Roberts et al. 2000), which were the case for these confocal measurements of sympathetic neurons. In addition, the SA estimates derived from confocal images depend on section thickness and pixel size so that, at low magnifications such as those used to capture the dendrites, the imaged object can be markedly distorted. Finally, neuronal SAs as large as derived from these images cannot be supported by our modeling, unless the electrical parameters are allowed to vary to unrealistic values. The requirements would be that \(C_m\) be well below 0.75 \(\mu\)Fcm\(^{-2}\), \(R_i\) be beyond 2,500 \(\Omega\)cm, and \(R_m\) be at least twice as high as in our study (Fig. 3).

We have taken \(R_i = 100 \Omega\)cm as the best estimate for mammalian neurons. However, if some of the dendrites of these neurons are densely packed with mitochondria, as recently noted for these (Gibbins et al. 1998) and other neurons (Surkis et al. 1996), effective \(R_i\) might be much higher, especially in the distal dendrites. This might explain some of the SA discrepancy above. If mitochondrial crowding were a factor, dendritic \(R_i\) would be important in determining the electrotonic attenuation of synaptic inputs.

Contribution of somatic shunt conductance

As mentioned above for the case of leaky dendritic membranes (London et al. 1999), higher \(R_m\) values were derived when a somatic shunt conductance was included in the models. However, the results provided no evidence either way to confirm or deny the presence of a shunt, as was the case in at least one previous study (Major et al. 1994). Some modeled and experimental data agree more closely if a somatic shunt conductance was incorporated (Clements and Redman 1989; Thurbon et al. 1994, 1998). A somatic shunt might result if the somatic \(R_m\) was intrinsically lower than the dendritic \(R_m\) or if there was a real but artifactual shunt produced by insertion of the microelectrode. Any microelectrode shunt conductance would probably have to include a transmembrane current, rather than just a leak through a hole surrounding the microelectrode, because the latter would be expected to have had a larger effect on RMP than was observed (Clements and Redman 1989; Pongracz et al. 1991; Staley et al. 1992). Sympathetic neurons have similar firing rates when recorded either intracellularly (McLachlan et al. 1997) or extracellularly (Habler et al. 1994) in vivo, suggesting that RMP and the effectiveness of synaptic inputs are not significantly changed by microelectrode penetration.

It has been suggested that a somatic shunt conductance is not present in whole cell recordings made with patch electrodes (Thurbon et al. 1998). However, dialysis through whole cell patch electrodes has been shown to increase cell input resistance, probably by reducing second messenger systems that control K\(^+\) channels (Robinson and Cameron 2000). Similarly, in vivo whole cell recording of sympathetic postganglionic neurons (Gola and Niel 1993) demonstrated "pacemaker" firing properties that have never been observed with either microelectrodes (Cassell et al. 1986) or extracellular recording techniques in vivo (e.g., Jänic et al. 1991). In fact, the reported
values for passive electrical properties are quite similar when recorded with either high resistance “sharp” microelectrodes or patch pipettes, at least for sympathetic neurons (cf. Keast et al. 1993; Vanner et al. 1993), provided allowance is made for the absence of dendrites following dissociation and the neurons are derived from animals of comparable age. Finally, when K⁺ channels in these neurons are pharmacologically blocked, the input resistance measured with an intracellular microelectrode can rise as high as \( R_N = 1 \ \text{G} \Omega \) (Göransson et al. unpublished observations). If \( g_{\text{shunt}} \) is considered to be entirely due to leakage around the microelectrode rather than being a transmembrane conductance, blocking K⁺ channels should not change the shunt. \( G_N = 1 \ \text{nS} \) implies that \( g_{\text{shunt}} < 1 \ \text{nS} \). Thus, while the possibility of microelectrode impediment artifact cannot be discounted, it seems likely that such artifacts were small in the present experiments.

Whereas the physical effects of impalement are unlikely to differ systematically between classes of sympathetic neuron, \( g_{\text{shunt}} \) might differ if the cell bodies of neurons of each class express different resting leak conductances as our data suggest. In the models, if \( g_{\text{shunt}} \) was allowed to differ between classes, it became possible to derive a uniform dendritic architecture. Nonetheless, the three neuronal phenotypes expressed different resting leak conductances as our data suggest. For LAH neurons, \( g_{\text{shunt}} = 1–2 \ \text{nS} \) for Ph neurons, and \( g_{\text{shunt}} < 1 \ \text{nS} \) for T neurons (Fig. 6). This is consistent with a somatic shunt of \( 1–2 \ \text{nS} \) (\( R_{\text{shunt}} \) = 0.5–1 \ \text{G} \Omega \) in all neurons and an additional shunt of about 3 \( \text{nS} \) on the LAH cell bodies due to activation of the \( \text{Ca}^{2+} \)-activated conductance. Activation of an additional \( \text{K}^{+} \) conductance by the influx of \( \text{Ca}^{2+} \) at the time of impalement would be expected to hyperpolarize the LAH neurons, but their RMPs are not more negative than those of other classes of neuron (Davies et al. 1999). Overall, it seems more likely that there is a real difference in the density of passive leak channels between the three neuronal phenotypes.

**Conclusion**

The present modeling study has revealed another distinct difference in the electrophysiological characteristics of the three phenotypes of sympathetic neuron. Not only are there different populations of voltage- and \( \text{Ca}^{2+} \)-dependent conductance present in each class, but characteristic nonuniformities in the distribution and type of membrane channels (including passive leak channels) contribute to the resting conductance. This property is of prime importance for the integration of subthreshold synaptic responses. However, it is clear that work in rat SCG activity in vivo arises almost exclusively from large suprathreshold (“strong”) preganglionic inputs (McLachlan et al. 1997), and summation of subthreshold (“weak”) inputs is rare. The same is probably true for LAH neurons that also receive a large strong input (McLachlan and Meckler 1989). However, T neurons in preganglionic inputs to the intestine which must summate, together with relatively small amplitude responses arising from the preganglionic inputs, to activate the cells (McLachlan and Meckler 1989). These neurons also receive peptidergic inputs during distension of the intestine which modulate resting conductance and amplify the fast inputs (Kreulen and Peters 1986). Combined with the higher effective \( R_m \), this synaptic repertoire provides T neurons with considerable flexibility in their mechanisms for integration. Thus the different classes of sympathetic neuron are well designed to operate variously as relays (strong inputs to neurons with relatively low \( R_m \)) or by integration (modulation of summed weak inputs and a high \( R_m \)).

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