Electrotonic Structure of Motoneurons in the Spinal Cord of the Turtle: Inferences for the Mechanisms of Bistability

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Understanding how voltage-regulated channels and synaptic membrane conductances contribute to response properties of neurons requires reliable knowledge of the electrotonic structure of dendritic trees. A novel method based on weak DC field stimulation and the classical method based on current injection were used to obtain two independent estimates of the electrotonic structure of motoneurons in an in vitro preparation of the turtle spinal cord. DC field stimulation was also used to ensure that the passive membrane properties near the resting membrane potential were homogeneous. In two cells, the difference in electrotonic lengths estimated with the two methods in the same cell was 6 and 9%. The majority of dendritic branches terminated at a distance of 1 electrotonic unit from the recording site. The longest branches reached 2A. In the third cell, the difference was 36%, demonstrating the need to use both methods, field stimulation and current injection, for reliable measurements of the electrotonical structure. Models of the reconstructed cells endowed with voltage-dependent conductances were used to explore generation mechanisms for the experimentally observed hysteresis in input current-voltage relation of bistable motoneurons. The results of modeling suggest that only some dendrites need to possess L-type calcium current to explain the hysteresis observed experimentally and that dendritic branches with different electrotonical lengths can be bistable. Independent bistable behavior in individual dendritic branches can make motoneurons complex processing units.

INTRODUCTION

This paper deals with the well-known problem of defining the electrotonic image of a nerve cell. Electrotonic measurements provide necessary parameters for complex models of neurons and serve as a basis for understanding the functional role of linear and voltage-dependent membrane properties. For this reason, it is necessary to provide reliable estimates of the electrotonical parameters. Electrotonic measurements have been performed in morphologically reconstructed neurons of different types (Clements and Redman 1989; Fleshman et al. 1988; Major et al. 1994; Rapp et al. 1994; Thurbon et al. 1994, 1998). Unfortunately with the methods used, it was not possible to check for homogeneity of the passive membrane properties. Thus it was necessary either to assume homogeneity or include a fourth unknown parameter to account for the difference between somatic and dendritic membrane properties. However, recordings with two electrodes in a single cell have suggested that dendritic membrane properties can also be intrinsically heterogeneous even near the resting potential (Stuart and Spruston 1998).

Recently methods based on stimulation with weak electrical DC fields were proposed for detecting membrane heterogeneity and injury shunts and for estimating the electrotonic structure of neurons (Svirskis et al. 1997a,b). If the passive membrane resistance of a neuron is homogeneous, then the transient in response to an applied DC field has no characteristic shape peculiarities and develops faster than the response to current injected through the recording electrode (Svirskis et al. 1997b). This transient depends only on membrane time constant, ρ, and electrotonical length constant, λ (Svirskis et al. 1997a), allowing for a more reliable estimation of the electrotonical structure of neurons. Since the check for homogeneity of the passive properties can be done during experiments, only cells fulfilling these criteria were chosen for electrotonical measurements. The combination of DC field method with the classical current injection method can increase the reliability of electrotonical measurements significantly.

In the present study, the electrotonic parameters were found for three motoneurons in slices of the turtle spinal cord. Two independent estimates of the electrotonic structure were obtained for each cell from responses to weak DC field stimulation and current pulse injection. An acceptable difference between parameters estimated with different methods in two cells suggests that errors introduced by staining procedures and reconstruction were inessential.

Because numerous potential-dependent currents are present in the neuronal dendrites, electrotonical structure alone can provide only a basis for further exploration of how synaptic input is processed. Models of reconstructed motoneurons were used to explore the generation of nonlinear properties due to potential-dependent inward current observed previously (Hounsgaard and Mintz 1988). It is known that part of this inward current is generated in the dendrites of motoneurons in turtles and cats (Hounsgaard and Kiehn 1993; Lee and Heckman 1996, 1998a). In voltage clamp, turtle and cat motoneurons show hysteresis in their input current-voltage relation (I-V) (Lee and Heckman 1998b; Schwindt and Crill 1980;...
Svirskis and Hounsgaard 1998). The presence of the hysteresis during very long voltage ramp stimulation (Lee and Heckman 1998b) suggests that dendrites are bistable (Gutman 1984; Jack et al. 1983) as theoretical studies had anticipated (Buttimas and Gutman 1978, 1981) from early experimental findings (Schwindt and Crill 1977, 1980). Qualitatively, the hysteresis can be explained by the relatively weak electrical coupling between soma and distal dendrites that allows distal inward current to be activated even when the proximal dendrite is relatively hyperpolarized. Here we explored how strong and where the inward current should be to generate hysteresis as observed experimentally.

Our models suggest that dendrites with different electrotonic length can be bistable and that only a fraction of the dendrites have to be nonlinear to generate the hysteresis observed experimentally. The results also show that a simple equation derived previously for nonlinear cables can be used to predict the behavior of more realistic models.

**Methods**

**Methods for the experimental procedures**

Transverse sections of the lumbar spinal cord were obtained from turtles (*Pseudemys scripta elegans*) deeply anesthetized with pentobarbitone (100 mg/kg) (Hounsgaard et al. 1988). The medium contained (in mM) 120 NaCl, 5 KCl, 15 NaHCO3, 20 glucose, 2 MgCl2, and 3 CaCl2, 6-Cyano-7-nitroquinazoline-2,3-dione (CNQX; 40 μM; Tocris Cookson, Bristol, UK) was applied to block excitatory synaptic potentials. For experiments a section of the cord, 0.5-mm thick, was placed in the recording chamber between two silver-chloride electrodes (see Fig. 2A in Svirskis et al. 1997b) used to establish an extracellular DC field.

For recordings, patch electrodes were pulled from borosilicate glass tubes with an outer diameter of 1.5 mm and an inner diameter of 0.86 mm. Electrodes were filled with 125 mM potassium glutonate, 9 mM HEPES, and 1% bicocytine; pH was adjusted to 7.4 with KOH. During whole cell recording, voltage transients were generated by injecting a current pulse of 0.3–1.0 nA for 1 ms through the recording electrode or by applying an extracellular current pulse of 1 μA between field electrodes for 150 ms (Svisrskis et al. 1997b). The response to the DC field stimulation depends on the direction of the field. Since the transmembrane potential induced by the electric field is largest in direction of the applied field, the shape of the evoked transient reflects electrotonic structure of the dendrites oriented mainly in direction of the field. The DC field was applied in the lateral direction because turtle spinal cord motoneurons have their physically longest dendrites oriented laterally (Ruigrok et al. 1985). To reduce noise, 256 sweeps were averaged on a HIIOKI digital oscilloscope (Hioki E. E. Corp., Nagano, Japan) and fed to a computer for later analysis.

After all measurements were accomplished, the electrode was withdrawn from the cell, and the extracellular potential, induced by the same field step stimulus, was recorded, averaged and subtracted from the intracellular potential to get the transmembrane potential. The electrotonic measurements. The dimensions of the slice were measured and correct for shrinkage due to histological procedures. The position of the electrode in relation to the borders of the slice was obtained.

Electrotonic estimates were obtained from three motoneurons recorded with patch electrodes. All responses to the field step had no characteristic shape peculiarities and decayed faster than the response to current injection (Fig. 2), indicating homogeneity of passive membrane resistance (Svirskis et al. 1997a,b). The responses to current pulses of opposite sign were anti-symmetric, showing that membrane properties were linear within the range of the response amplitudes.

**Staining and reconstruction**

Standard procedures were applied to stain biocytin-injected neurons (Horikawa and Armstrong 1988). In brief, slices were kept in fixative overnight, then washed in phosphate buffer and treated with H2O2. After removal of hydroxy peroxide, slices were incubated with ABC complex and 1% Triton overnight. After wash, DAB treatment was used to visualize the stained neurons. Nickel salt was used to get almost black colored staining. To dehydrate slices, the incubation solution was gradually changed to pure ethanol. Then slices were cleared in xylene and mounted in Permount.

For reconstruction, semi-automatic NeuroLucida software and hardware were used with Zeiss microscope. Water-immersed objective allowed to define the diameter with a precision of 0.3 μm in stained motoneurons. Soma was reconstructed as a part of the dendritic tree. The slice contour was outlined to define the shrinkage, which was 1.3–1.6 times, and to set the field direction in relation to reconstructed neuron. The position of the recording electrode was estimated after the coordinates were corrected for the shrinkage. In two motoneurons, this position hit the soma region, whereas in one, it coincided with the proximal dendrite (Fig. 3). Diameters of the dendritic branches were not corrected for the shrinkage, which could cause either the reduction or enlargement of the diameters depending on the intracellular contents of the dendrites (see Major et al. 1994) (see also DISCUSSION).

**Calculations for electrotonic measurements**

Homogeneous passive membrane properties were assured experimentally by using DC field stimulation as described in the preceding text. For the description of the linear responses of neurons with homogeneous membrane properties, we therefore used a set of electrotonic parameters: membrane time constant, τ = Rm/Cm; electrotonic length constant, λ = (RmμaRm/σ1/2); and characteristic resistance (resistance of semi-infinite cable), Rc = (RmμaRm/σ1/2), where Rm is the specific membrane resistance, Cm is the specific capacitance, and μa is the specific intracellular resistance. Here, μa is the area of a cross-section of the cable and σ is the perimeter. The apparent diameter, D, is a morphologically measured quantity, σ ≡ D and a ≡ D2 with constant proportionality coefficients throughout the dendritic tree on a macroscopic scale (Alaburda and Gutman 1996). Thus λ and Rm were constant over the same scale. Because λ ∼ D1/2 and Rc ∼ D−3/2, it is necessary only to know values of parameters for the single particular diameter to define the response to the current and DC field stimulation of the complete dendritic tree. For this purpose, we used constants λ1 and Rm, which are defined as a electrotonical length and characteristic resistance for a homogeneous dendritic segment with an apparent diameter, D = 1 μm (Svisrskis et al. 1997a). For any dendritic segment with a diameter of D μm, A and Rm were found by multiplying λ1 and Rm by dimensionless number equal to D1/2 and D−3/2, respectively.

The electrotonic parameters, τ, λ1, and Rm, were estimated by comparing experimental and simulated transients after current pulse injection and stimulation with DC field. Calculations in models of the reconstructed motoneurons were accomplished using Fourier transformation. The results were checked by solving the system of ordinary
differential equations directly for the compartmental model of reconstructed motoneurons using the method of Cranck-Nicholson (Press et al. 1992). The same system of equations was also solved as a matrix equation for the eigenvalues, \( \tau \), and eigencoefficients, \( E_n \). Here, \( I(t) \) is the harmonic component of the current injected through the microelectrode; \( \theta \) is the cyclic frequency; \( \lambda \) is the length constant for harmonic potentials, \( \lambda = \lambda_f/(1 + j0\theta) \), where \( j \) is imaginary unit; \( \Omega \) is the characteristic impedance; \( \Omega = R_{\text{ch}}/(1 + j0\theta) \) for the segment with \( D = 1 \mu m \), and \( D_{\text{ch}} \) is the dimensionless number equal to the diameter, expressed in micrometers, of the \( n \)th proximal segment. If the transmembrane potential change is induced only by the field then the harmonic component

\[
V(\theta) = \frac{\Omega}{\lambda} I(\theta) \left( \sum_n D_n^2 A_n(\lambda) \right)
\]

Here \( I(\theta) \) is the harmonic component of the current injected through the microelectrode; \( \theta \) is the cyclic frequency; \( \lambda \) is the length constant for harmonic potentials, \( \lambda = \lambda_f/(1 + j0\theta) \), where \( j \) is imaginary unit; \( \Omega \) is the characteristic impedance; \( \Omega = R_{\text{ch}}/(1 + j0\theta) \) for the segment with \( D = 1 \mu m \), and \( D_{\text{ch}} \) is the dimensionless number equal to the diameter, expressed in micrometers, of the \( n \)th proximal segment. If the transmembrane potential change is induced only by the field then the harmonic component

\[
W(\theta) = E \cdot \left( \sum_n D_n^2 B_n(\lambda) / \left( \sum_n D_n^2 A_n(\lambda) \right) \right) \left( \sum_n D_n^2 A_n(\lambda) \right)
\]

Here, \( E \) is the strength of the DC field. Coefficients \( A_n \) and \( B_n \) were calculated using recursive equations

\[
A = \lambda \sqrt{D} \left[ \left( \frac{\lambda}{\lambda} \right) \left( \frac{\cos \left( \frac{\theta}{\lambda} \right) + \lambda G \sqrt{D} \sin \left( \frac{\theta}{\lambda} \right) }{\frac{\sin \left( \frac{\theta}{\lambda} \right) + \lambda G \sqrt{D} \cos \left( \frac{\theta}{\lambda} \right) }{\lambda} } \right) \right]
\]

\[
B = \lambda \sqrt{D} \left[ F \left( \frac{\sin \left( \frac{\theta}{\lambda} \right) + \lambda G \sqrt{D} \cos \left( \frac{\theta}{\lambda} \right) }{\lambda} \right) - \lambda \cos \left( \frac{\theta}{\lambda} \right) \right]
\]

where \( X \) is the length of the segment, \( \alpha \) is the angle between the segment and the field. For terminal segments, \( G = 0 \), \( F = \cos \alpha \). For all other segments, \( G = \sum D_n^2 A_n D_n \) and \( F = \cos \alpha + \sum D_n^2 B_n A_n D_n \). The sum is carried out for segments adjacent to the distal end of the considered segment, \( D_n \) are dimensionless numbers equal to the diameters, expressed in micrometers, of adjacent segments, \( D_n \) of the more proximal segment considered. Starting the calculation from terminal segments, coefficients \( A_n \) and \( B_n \) were found for the proximal segment of every dendrite emerging from the recording point. In our reconstruction of the neurons, the soma was represented as a part of the dendritic tree.

From Eqs. 1 and 2 and after inverse Fourier transformation, it is evident that in the case of current injection the response of transmembrane potential in any point of the neuron depends on \( \lambda_1 \), \( \tau \), and linearly on \( R_{\text{mem}} \). In the case of field stimulation, the response depends only on \( \lambda_1 \) and \( \tau \). Another advantage of using the electrotonic set of parameters is that the solution for the complete dendritic tree does not require specifying the shape of the dendritic cross-section. Solution dependence on the shape is hidden in the electrotonic parameters.

Calculations for nonlinear models

To analyze the mechanisms responsible for the experimentally observed nonlinear properties of motoneurons, we added L-type calcium channel conductance and potassium delayed rectifier conductance in the membrane models of the reconstructed neurons. Parameters and equations for these conductances were similar to those used previously in models of bistable motoneurons (Booth et al. 1997). Calcium current \( I_{\text{ca}} = G_{\text{ca}} [m(V - V_{\text{ca}}) - m( - 65 - V_{\text{ca}}) - \alpha \mu m/s] \); calcium channel activation variable, \( m \), was governed by the equation \( \frac{dm}{dt} = (m_{\infty} - m)/\tau_{\text{ca}} \), where \( m_{\infty} = \psi(1 + \exp(V - \theta_1)/k_1) \), \( V_{\text{ca}} = 40 \text{ mV}, \tau_{\text{ca}} = 20 \text{ ms}, \theta_1 = -35 \text{ mV}, k_1 = -5 \text{ mV} \). The conductance parameter, \( G_{\text{ca}} \), was used as a variable parameter. Since the activation kinetics of the K- delayed rectifier channel is very fast compared with the stimulus protocol used (see following text), the activation was modeled as instantaneous. Thus potassium current \( I_K = G_K n^4 (V - V_{\text{K}}) \), where \( n = 1/[1 + \exp(V - \theta_2)/k_2] \), \( V_{\text{K}} = -90 \text{ mV}, \theta_2 = -30 \text{ mV}, k_2 = -12 \text{ mV}, \) and \( G_K = 3 R_{\text{mem}} \). Input I-Vs for the reconstructed neurons were computed by simulating voltage clamp as triangular ramps of the soma voltage. The speed of the ramp was made 0.005 mV/ms to get almost stationary input I-V. Calculations were performed by directly solving the system of ordinary differential equations for the compartmental models of reconstructed motoneurons.

RESULTS

First of all, we illustrate that the responses to intracellular current injection and field stimulation are independent, i.e., are not linearly transformable. For this purpose, we calculated the eigenvalues and eigencoefficients of the system of equations describing reconstructed motoneurons in both cases of stimulation (see METHODS). As seen in Fig. 1 the eigenvalues are the same for both types of responses except for the absence of the component with the membrane time constant in response to DC field stimulation (Svirskis et al. 1997b). However, the coefficients are very different indicating that transients after current injection and field stimulation are independent. This shows that the two methods provide independent measures of the electrotonic structure of neurons and that they therefore also provide a mutual validation.

The transients in neurons with homogeneous passive properties depend on the electrotonic parameters in a way that allows the parameters to be estimated one by one. First, by using the response to injected current pulses, the membrane time constant, \( \tau \), was estimated from the exponentially decaying part of the transient (Fig. 2A). Values of \( \tau \) varied from 10 to 29 ms for the three motoneurons (see Table 1). Since the transient after a current pulse is proportional to \( R_{\text{mem}} \) (see METHODS), the value of this parameter was made equal to 1 M\( \Omega \) when calculating the response of the model of reconstructed motoneurons. Only \( \lambda_1 \) value was changed to achieve the best correspondence between experimental and calculated transients. The fitting was done by dividing the experimentally obtained transient by the calculated transient; this gave the \( R_{\text{mem}} \) value for each point of the transient (Fig. 2B, bottom). Because the calculated transient should be proportional to the \( R_{\text{mem}} \) sought, large variance of \( R_{\text{mem}} \) values at the points of the transient indicates a poor fit to the experimental data. Usually
brane time constant, \( t \) (estimated from the field stimulation), and characteristic resistance, \( R \).

### TABLE 1. Ohmic parameters of spinal cord motoneurons

<table>
<thead>
<tr>
<th>Neuron</th>
<th>( \tau ), ms</th>
<th>( \lambda_1 ), ( \mu )m</th>
<th>( R_{1\infty} ), M( \Omega )</th>
<th>( C_{\text{mem}} ), ( \mu )F/cm(^2)</th>
<th>( R_{\text{in}} ), k( \Omega ) \cdot cm(^2)</th>
<th>( R_i ), ( \Omega ) \cdot cm</th>
<th>( \lambda_{1F} ), ( \mu )m</th>
<th>( R_{\text{in}} ), M( \Omega )</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2206</td>
<td>29</td>
<td>800</td>
<td>1030</td>
<td>1.1</td>
<td>26</td>
<td>100</td>
<td>850</td>
<td>46</td>
</tr>
<tr>
<td>M0207</td>
<td>12</td>
<td>550</td>
<td>1150</td>
<td>0.6</td>
<td>20</td>
<td>160</td>
<td>350</td>
<td>28</td>
</tr>
<tr>
<td>M0607</td>
<td>10.5</td>
<td>600</td>
<td>750</td>
<td>0.7</td>
<td>14</td>
<td>100</td>
<td>550</td>
<td>16</td>
</tr>
</tbody>
</table>

The electrotonic set of parameters consists of membrane time constant, \( \tau \), electrotonic length constant, \( \lambda_1 \) (estimated from the current injection) and \( \lambda_{1F} \) (estimated from the field stimulation), and characteristic resistance, \( R_{1\infty} \). Length constants and characteristic resistance are for the cable with the diameter of 1 \( \mu \)m. The electric set of parameters: specific membrane capacitance, \( C_{\text{mem}} \), specific membrane resistance, \( R_{\text{mem}} \), specific intracellular resistance, \( R_i \), \( R_{\text{in}} \) stands for the input resistance.

and ranged from 550 to 850 \( \mu \)m (Table 1). The procedure described above simultaneously provided an estimate for the characteristic resistance, \( R_{1\infty} \) (see Table 1), which was calculated as an average of \( R_{1\infty} \) values for all points in the transient.

To validate the procedure used, we also calculated the average of squared difference between the experimental and the theoretical transients. In this case the calculated transients were multiplied by the estimated \( R_{1\infty} \) value. The estimates of \( \lambda_1 \) provided the minimal average of the squared difference (Fig. 2B, inset). The electrical parameters were then calculated assuming that the dendritic cross-section is circular. The specific membrane capacitance, \( C_{\text{mem}} \), ranged from 0.6 to 1.1 \( \mu \)F/cm\(^2\), specific membrane resistance, \( R_{\text{mem}} \), had values from 14 to 26 k\( \Omega \) \cdot \text{cm}^2, and cytoplasmic resistance, \( R_i \), ranged from 100 to 160 \( \Omega \) \cdot \text{cm} (Table 1).

To check the validity of measurements, we also estimated \( \lambda_1 \) by simulating the response of the models of reconstructed motoneurons to the field stimulation. This response depends only on \( \tau \) and \( \lambda_1 \) (see METHODS). Since the membrane time constant, \( \tau \), could be reliably estimated from the response to current injection (Fig. 2A), we used it in these simulations as a known parameter. We varied the value of \( \lambda_1 \) and found the estimate which produced the minimum of the average of the squared difference between experimental and calculated transients (Fig. 2C, inset). In two cells the difference between estimates of \( \lambda_1 \) obtained by the two methods in the same cell was 6 and 9%, showing that possible errors due to histological procedures were inessential. In the third cell, the difference was 36%, demonstrating the necessity to use current and field stimulations to ensure the reliability of the measurements.

Knowing the electrotonic length constant, \( \lambda_1 \), defined for the diameter of 1 \( \mu \)m allows calculating electrotonic length constant for any dendritic segment with a diameter of \( D \) \( \mu \)m as \( \lambda = \lambda_1 \cdot D^{1/2} \) (see METHODS). Surprisingly, despite very different membrane time constants between neurons the electrotonical structure of the dendritic trees did not vary much. The majority of the branches terminated at the electrotonical distance around 1 \( \lambda \) from the recording site (Fig. 3) for all three neurons. However, some branches reached as far as 2 \( \lambda \) (Fig. 3B).

Since dendrites also possess voltage-dependent conductances, the electrotonic structure provides just a starting point for the exploration of synaptic integration. In motoneurons, dendritic inward current mediated by L-type calcium channels is responsible for plateaus and hysteresis in the input \( I-V \) (Hounsgaard and Kiehn 1993; Lee and Heckman 1998b; Schwident and Crill 1980; Svirstis and Hounsgaard 1998). To investigate the generation of hysteresis in input \( I-V \), reconstructed neurons were used to create nonlinear models with...
L-type calcium and potassium delayed rectifier conductances (see METHODS).

Input \(I-V\)s were calculated using the complete model of reconstructed motoneuron m2206 (Fig. 4A). To mimic experiments, a slow triangular voltage-clamp ramp at the soma was used as a stimulus for these calculations. Hysteresis appeared in the \(I-V\) plot when the conductance of calcium current, \(G_{\text{ca}}\), was increased to \(1.55/R_{\text{m}}\). With the further increase of calcium conductance, \(G_{\text{ca}} = 2.95/R_{\text{m}}\), the hysteresis became broader and deeper (not shown). However, in experiments, the \(I-V\) hysteresis observed is not very deep. Possibly, only some dendrites have potential-dependent inward currents. As a check, we endowed only the third dendrite of the motoneuron (Fig. 3) with the voltage-dependent conductances while the other two dendrites were left only with the passive leakage current. In this case, the simulated \(I-V\) plot (Fig. 4A) became more similar to the experimentally observed plot (Svirskis and Hounsgaard 1997, 1998).

The mechanisms of hysteresis could be understood from the distribution of membrane potential in the dendritic tree (Fig. 4B) when hysteresis is narrow, i.e., \(G_{\text{ca}} = 1.55/R_{\text{m}}\). When the soma voltage was decreasing during the falling phase of the triangular ramp, in some interval of the soma voltage the longest branches were more depolarized than the cell body due to activation of the persistent inward current and weak coupling to the soma (Fig. 4B). The depolarization of the terminal dendrites was absent in the same interval of clamped soma voltage when voltage ramp was rising because the inward current was not yet activated. Thus this bistability of branches is reflected as hysteresis in input \(I-V\). The depolarization of the same two branches also caused the last jumps in input \(I-V\) for the larger value of \(G_{\text{ca}} = 2.95/R_{\text{m}}\) (Fig. 4A), which made hysteresis broader and induced dendritic bistability (Fig. 4B) in shorter branches. Thus in case of broad hysteresis observed under experimental conditions the dendrites with different electrotonic length could be bistable. As the number of bistable branches increases, the variety of responses of motoneuron to synaptic input also increases (Gutman 1991).

Interestingly, the value of calcium conductance, \(G_{\text{ca}} = 1.55/R_{\text{m}}\), which induced hysteresis in the model of reconstructed motoneuron could be estimated by using a simple equation. For cables, a mathematical relation defines whether hysteresis is present in the stationary input \(I-V\): the stationary negative slope-conductance, \(S_N\), in membrane \(I-V\) (Fig. 4C) should be \(S_N > \pi^2/4R_mL_{\text{cable}}^2\), where \(L_{\text{cable}}\) is cable electrotonic length (Baginskas et al. 1999; Gutman 1991; Jack et al. 1983). For the cable with the electrotonical length \(1\lambda\), \(S_N = 2.47\). To achieve such a slope in membrane \(I-V\), the calcium conductance, \(G_{\text{ca}}\), should be equal to \(1.55/R_{\text{m}}\), according to the equations in METHODS. In agreement with this estimate only the dendritic branches longer than \(1\lambda\) were bistable in the case of \(G_{\text{ca}} = 1.55/R_{\text{m}}\) (Fig. 4B). This example shows that mathematical
Electrotonic measurements are prone to errors. Tissue dimensions are changed by the histochemical procedures employed for fixation and staining. In our experiments, shrinkage in tissue dimensions was 1.3–1.6 times. While the lengths of dendrites were corrected, we have chosen not to change the diameter values measured because the shrinkage could cause either reduction or increase of the dendritic diameters (see Major et al. 1994). The change in the diameters is important for estimates of the electrical parameters $R_m$, $C_m$, $R_i$. However the electrotonical structure is less sensitive to global changes in diameters. For example, increase of all diameters by $r$ times causes the reduction of the electrotonical length by the factor $r^{1.5}$ if $\lambda_1$ is kept constant. But this results in faster transients, which require a decrease of $\lambda_1$ to fit the experimental transient. We estimated $\lambda_1$ values for all three neurons after diameters were corrected for shrinkage. The reduction of $\lambda_1$ was 500/600 = $1/(1.44)^{1.5}$, 675/800 = $1/(1.4)^{1.5}$, and 500/550 = $1/(1.2)^{1.5}$ for the shrinkage of 1.6, 1.3, and 1.3 respectively in three cells. Thus the electrotonical structure would not change significantly if diameters were corrected for shrinkage.

The other source of possible error is the morphological reconstruction. We evaluated the electrotonical structure after inducing a significant reduction in diameter of a compartment near the recording site. In this case, the estimates of $A$ obtained by injection of current pulses and by DC field stimulation differed several times. Since the experimental estimates we obtained differ by only 6 and 9% in two cells, we can be sure that the morphological reconstruction was without major errors.

The only modest correspondence between the estimates of the electrotonical structure in cell m2027 could be explained by the heterogeneity in field strength, which equaled 15% and was the largest among the three cells, distortion of the response to the current impulse by pipette capacitance, and/or heterogeneous shrinkage. At this stage, we cannot distinguish between these explanations. Note, however, that for this cell the value of $C_m$ is smaller and the value of $R_i$ considerably larger than for the other two cells.

The calculated values of eigencoefficients (Fig. 1) show a striking difference between responses to the field stimulation and current injection. The eigencoefficients of the fast components are very small in the response to DC field stimulation. Hence the decay rate of the whole transient is a satisfactory reflection of the electrotonical lengths of dendritic branches, which are oriented in the direction of the field. This result validates the DC field stimulation as a method for the estimation of the equivalent electrotonical length of the dendritic tree (Svirskis et al. 1997a). Cell m2206 is particularly good for illustration because the evolution of the response evoked by the DC field depends mainly on two exponents (Fig. 1). The slowest time constant of the transient, $\tau_c = 5.6$ ms, and the membrane time constant, $\tau = 29$ ms, allow us to calculate an estimate of the tip-to-tip electrotonical length $L$ in the field direction. According to the classical Rall equation: $L = \pi l (2\tau_c/\tau - 1)^{1/2} \approx 1.5$ and is much shorter than $L$ for the two other cells. The estimation fits quite well to the electrotonical structure if we notice that cell m2206 has rather short laterally oriented dendrites and long $\lambda$ (Fig. 3A).

As shown here and elsewhere (Svirskis et al. 1997a,b), DC field stimulation offers several advantages for electrotonical measurements. The essence of the method is that during weak...
DC field stimulation the total current passing the membrane of a neuron equals to zero (Svirskis et al. 1997b)—there are no net current sinks or sources. Although we used the extracellular current to create a potential gradient for polarizing neurons, other methods could be used as well. Recordings from the same neuron with two independent electrodes have proved feasible (Stuart and Spruston 1998; Stuart et al. 1993). Electrotonic measurements can be obtained with this configuration keeping the total current flowing through the neuronal membrane zero by injecting current of equal amplitude but opposite polarity through the two electrodes. Also the check for homogeneity of the specific membrane resistance is the same as with the field stimulation.

Neglecting the uncertainties in correcting diameters and assuming circular cross-sections of dendritic branches, we have obtained electrical parameters for dendritic cables. The specific membrane resistance, $R_m$, was in the range from 14 to 26 $k\Omega \cdot \text{cm}^2$; specific membrane capacitance was from 0.6 to 1.1 $\mu\text{F/cm}^2$; and the specific cytoplasmic resistance was from 100 to 160 $\Omega \cdot \text{cm}$ (see Table 1). The values of these parameters are in the range observed in other studies. Specific membrane capacitance was estimated to be from less than 1 $\mu\text{F/cm}^2$ (Major et al. 1994) to more than 2 $\mu\text{F/cm}^2$ (Rapp et al. 1994; Thurbon et al. 1998). Estimates of other parameters varied even more. Specific membrane resistance was estimated to be from tens of $k\Omega \cdot \text{cm}^2$ (Larkman et al. 1992; Meyer et al. 1997; Thurbon et al. 1994, 1998) to hundreds of $k\Omega \cdot \text{cm}^2$ (Major et al. 1994; Rapp et al. 1994). Specific cytoplasmic resistance had values from less than 100 $\Omega \cdot \text{cm}$ (Thurbon et al. 1994, 1998) to several hundred $\Omega \cdot \text{cm}$ (Larkman et al. 1992; Major et al. 1994; Meyer et al. 1997; Rapp et al. 1994). The huge variation of the estimates may be attributed to the reasons outlined in the preceding text and/or to biological differences between cell types. The electrotonic set of parameters, $\tau, \lambda_1, \text{and } R_{I\text{ext}}$, has several advantages over the electrical set, $C_m, R_m$, and $R_I$. First, each parameter has its own functional meaning for the response of dendritic cables. Second, the estimates are less vulnerable to unverified assumptions, i.e., circular cross-section of the dendrites. Third, the parameters can be estimated one by one, which improves the reliability of estimates.

In motoneurons, voltage-dependent currents together with passive conductance shape the response to synaptic input. Persistent dendritic inward current mediated by L-type calcium channels is responsible for generation of plateau potentials in current-clamp mode (Hounsgaard and Kiehn 1993;Lee and Heckman 1996, 1998a) and for hysteresis in voltage-clamp mode (Lee and Heckman 1998b; Schwindt and Crill 1980; Svirskis and Hounsgaard 1997, 1998). In this study, we used a model with L-type calcium current to show that dendrites with different electrotonic length could be bistable in case of the experimentally observed broad hysteresis in input $I-V$. Although only three motoneurons were reconstructed in the present study, previous morphological investigations (Ruigrok et al. 1984, 1985) showed that short terminal dendritic branches in abundance is a general characteristic for turtle motoneurons.

In our model, membrane conductances were homogeneously distributed in the dendrites. This may not be true in real dendrites. Because the dendrites are not very long electrotonically, membrane potential changes in space are smooth (Fig. 4B) in our case of slow currents and slow somatic voltage-clamp ramps. Thus effects of any possible heterogeneities of potential dependent conductances would be smoothed over entire dendritic branches. In this case, negative slope in membrane $I-V$, which defines when dendritic bistability could occur, would represent an average of the membrane conductances over the dendritic branches.

As demonstrated here, even in branching dendrites, hysteresis in input $I-V$ depends only on electrotonic length of dendrites and the maximal negative slope in membrane $I-V$ (Fig. 4C). Consequently the inferences made do not depend on the detailed nature of persistent inward current and should be readily applicable to other types of motoneurons with observed bistability (Hsiao et al. 1998; Lee and Heckman 1998b; Rekling and Feldman 1997). Because of the independence of hysteresis on particular membrane mechanisms, we did not include other channels, like N-type calcium channel, calcium-sensitive potassium channel etc., in the model, although these channels are known to be present in turtle motoneurons. These currents could influence the temporal phenomenology, but they would not change our qualitative findings regarding hysteresis during very slow voltage ramps.

Very slow kinetic properties of inward currents could, however, have profound influence. A slow depolarization induced facilitation of inward current, possibly by changing voltage sensitivity of this current, was observed in motoneurons (Bennett et al. 1998; Svirskis and Hounsgaard 1997). An inward current with shifting voltage sensitivity due to slow facilitation has steep potential dependence and, accordingly, could increase the negative slope in membrane $I-V$ and cause hysteresis in input $I-V$ of electrotonically short cables (Baginskas et al. 1999). The slowness of inward current kinetics also explains why jumps in hysteresis were not observed experimentally. Thus it is very probable that dendritic branches with different electrotonic lengths can be bistable in turtle motoneurons.

In turtle motoneurons the persistent inward current is also modulated by neurotransmitters via metabotropic pathways (Svirskis and Hounsgaard 1998). Facilitation of persistent inward current by bath-applied agonist leads to bistability in current clamp and broad hysteresis in voltage clamp (Svirskis and Hounsgaard 1998). In contrast and in agreement with modeling results, focal synaptic facilitation of dendritic inward current merely increases excitability in current clamp mode and only induces narrow hysteresis in voltage clamp (Delgado-Lezama et al. 1997, 1999).

In conclusion, potential-dependent inward current causing bistability independently in numerous dendritic branches increases the richness of synaptic processing and makes motoneurons complex processing units (Gutman 1991).

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