Passive Electrical Properties of Ventral Horn Neurons in Rat Spinal Cord Slices

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Thurbon, David, Hans-R. Lüscher, Thomas Hofstetter, and Stephen J. Redman. Passive electrical properties of ventral horn neurons in rat spinal cord slices. J. Neurophysiol. 79: 2485–2502, 1998. Recordings were made from large neurons located in the ventral horn of transverse spinal cord slices from young rats (7–15 days). Whole cell recordings were made simultaneously with two electrodes from the soma of these neurons, visualized using infra-red differential interference contrast optics. Positive identification of motoneurons could not always be achieved. The response of a neuron to a brief pulse of current delivered by one electrode, and recorded by the other electrode, were matched optimally to responses of a compartmental model of the same neuron with an identical current pulse as input. The compartmental model was based on a reconstruction of the neuron, using Biocytin staining. The compartmental model had three free parameters: specific membrane capacitance ($C_m$), membrane resistivity ($R_m$), and cytoplasmic resistivity ($R_i$), all assumed to be uniform throughout the neuron. The experimental and model responses could be matched unequivocally for four neurons, giving $C_m = 2.4 \pm 0.5 \mu F/cm^2$, $R_m = 5.3 \pm 0.9 k\Omega/cm^2$, and $R_i = 87 \pm 22 \Omega/cm$. No somatic shunt was required. For the remaining six neurons, a less perfect fit (but still within 95% confidence limits) was indicative of nonhomogeneous membrane properties. The electrotonic length of uncut dendrites was $0.85 \pm 0.14 \lambda$. The results resolve the issue of a somatic shunt conductance for motoneurons, relegating it to a distinguishable ($at the 95\% confidence level$) from the ex-microelectrode impalement artifact. They are consistent with previous reports on the electrical compactness of motoneurons to steady state currents and voltages. However, the much higher value of $C_m$ (than the previously assumed $1 \mu F/cm^2$) implies much greater dendritic attenuation of fast synaptic potentials, and a much enhanced integrative response of motoneurons to synaptic potentials.

INTRODUCTION

Determination of the passive properties of neuronal membrane has been a goal of many investigations, commencing with Rall’s seminal papers (1959, 1960) on the motoneuron. A knowledge of the specific resistivity ($R_m$) and capacitance ($C_m$) of the membrane and the cytoplasmic resistivity ($R_i$) is fundamental to understanding the integrative properties of a neuron. This is true even for those neurons in which voltage-dependent membrane conductances are activated by small departures in membrane potential from the resting potential, as the passive characteristics serve as a baseline on which the active membrane properties must be superimposed.

An important development in the study of passive properties of neurons was to combine the electrophysiological measurements in a neuron with a reconstruction of that neuron’s morphology, first achieved by Lux et al. (1970). The advent of simple intracellular labeling methods (e.g., horseradish peroxidase, Biocytin R) has enabled a much wider use of simple recording techniques in vitro (Hamill et al. 1981). Optimized matching of recorded intracellular transients to transients generated in compartmental reconstructions of the same neuron allows a greater precision in parameter estimation than was possible using formulas based on equivalent cylinder assumptions. These progressive refinements in experimental techniques have brought increasing accuracy to the determination of $R_m$, $R_i$, and $C_m$.

In this investigation, we have continued to improve the recording technique by using two whole cell electrodes on the soma of ventral horn neurons, visualized using DIC-infrared microscopy (Stuart et al. 1993). This procedure minimizes the voltage artifact on the recording electrode by passing current through the second electrode. In one-third of the neurons investigated, we could adjust $R_i, C_m$, and $R_m$ in the compartmental models derived from the reconstructed neurons such that the transients generated in them were indistinguishable (at the 95% confidence level) from the experimental transients. For these neurons, $R_i = 87 \pm 22 \Omega/cm; C_m = 2.4 \pm 0.5 \mu F/cm^2$, and $R_m = 5.3 \pm 0.9 \Omega/cm^2$. No somatic shunt conductance or other nonuniformities in these parameters were required. In the other neurons, there was evidence of nonuniform membrane properties. The parameter values confirm previous conclusions on the electrical compactness of motoneurons for DC voltages (Clements and Redman 1989; Ulrich et al. 1994). However, the higher value of $C_m$ than the $1 \mu F/cm^2$ previously assumed for spinal motoneurons (Clements and Redman 1989; Fleschman et al. 1988) implies a greater dendritic attenuation of synaptic potentials than has been calculated previously.

METHODS

Experimental methods

Experiments and modeling were carried out in Canberra, the histological processing and reconstruction in Bern. The analysis is based on 12 cells that were chosen for their complete staining with Biocytin out of a total of 39 cells from which satisfactory electrophysiological data were obtained.

PREPARATION. Slices of lumbar spinal cord were obtained from 7- to 15-day–old Wistar rats of either sex. The rats were first anesthetized with halothane followed by urethan (10 g/100 ml, 0.1 ml per 10 g body wt ip) and cooled for 5–7 min in crushed...
ice. Thereafter they were decapitated, and the entire spinal cord dissected out by a ventral approach. Dural and pial membranes were removed under a dissecting microscope using fine forceps and scissors. During preparation the spinal cord was immersed in ice-cold artificial cerebrospinal fluid (ACSF) and gassed with a mixture of 95% O₂-5% CO₂. This ACSF consisted of (in mM) 113 NaCl, 3 KCl, 1 NaH₂PO₄, 25 NaHCO₃, 11 glucose, 2 CaCl₂, and 1 MgCl₂. The lumbar enlargement of the spinal cord then was isolated and embedded in Agar (1 g/60 ml ACSF), which was boiled, cooled to 40°C, and gassed before embedding took place. Transverse slices 300-μm thick were cut in ice-cold ACSF using a vibratome (Camden Instruments), transferred to a holding chamber, and allowed to recover for ≈1 h at 35°C. Thereafter the slices were kept at room temperature (20–24°C) for ≤6 h.

RECORDING. Single slices were transferred to a recording chamber and superfused at 1 ml/min with ACSF at 34 ± 2°C. The ACSF contained 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione, 100 μM picrotoxin, and 10 μM strychnine to block synaptic background activity. The slice was held in place in the recording chamber with a U-shaped platinum wire supporting parallel nylon threads (Edwards et al. 1989). One thread was placed across the central canal and another thread across the ventral border of the spinal cord, leaving most of the ventral horn free for electrode exploration. The neurons were visualized using Nomarski optics (×40 water immersion objective; Zeiss, Axioskop) and infrared illumination (Stuart et al. 1993). The image was magnified by a factor of 4 with a video adapter tube (Zeiss), recorded with an infrared sensitive vidicon camera (Hamamatsu, Model C2400, Japan), and displayed on a monitor. Whole cell recordings were made only from neurons with a cell body diameter >20 μm (the major diameter in the elliptic outline) in the hope that these neurons were most likely motoneurons (Takahashi 1990). However, a positive identification of the cell type was not possible, even after staining and reconstruction (see further). The electrodes were made from thick-walled borosilicate glass tubing (1 mm ID, 1.5 mm OD). They were filled with a solution of the following composition (in mM): 135 gluconic acid, 5 KCl, 5 Na₂ATP, 1.2 MgCl₂, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-(tetraacetic acid, 5 N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer to which 2% Biocytin (wt/vol; Sigma) was added, adjusted to pH 7.3 using KOH and osmolarity 280–290. Electrode resistances were between 5 and 8 MΩ.

The cell bodies were approached by two electrodes from the two opposing sides of the bath, each at an angle of ~30°. Two Axoclamp-2B amplifiers (Axon Instruments; Foster City, CA) were used. First a seal was established with both electrodes in voltage-clamp mode. After breaking into the cells with both electrodes, both amplifiers were switched to current clamp mode. A long (75 ms) hyperpolarizing current pulse of ~0.1 nA was injected through one electrode, and the bridge-balance carefully adjusted, using the resulting voltage transient recorded with the other electrode as a guide. This procedure then was repeated for the other electrode. Optimal capacitance compensation was assumed when the high-frequency noise increased without ringing. Membrane potential was held at ~65 mV (in all cases holding current and the 95% confidence band were computed after subtracting the average of the voltage response over the entire time course of the voltage response. Between 420 and 500 sweeps were used for averaging. Figure 1C shows a typical averaged transient as recorded with the voltage recording electrode together with its narrow 95% confidence band (420 sweeps). These voltage transients subsequently were used as the target response for estimating the parameter values for the model, one must ensure stationarity of the responses during the inspection visually for large noise contributions, and any contaminated records were rejected. Thereafter the sweeps were averaged and the 95% confidence band computed after subtracting the average voltage over the entire time course of the voltage response.

FIG. 1. Single sweep, subaverages, and average voltage transient. A: voltage response to ~0.5-nA, 480-μs current pulse from cell C3–1311, filtered at 10 kHz and digitized at 31.25 kHz. B: 4 sub-averages of 100 sweeps each superimposed. Near perfect superposition of the 4 subaverages suggests perfect stationarity of the voltage response over time. C: average voltage transient of 420 sweeps together with 95% confidence band.
period required to acquire the 500 responses. Comparing subaverages of 100 responses provides a simple test for stationarity. Figure 1B illustrates such a test. Four consecutive subaverages of 100 sweeps each are superimposed. The perfect superposition of all four subaverages suggests perfect stationarity.

In addition to the check for stationarity, care was taken to ensure that the injected short hyperpolarizing current did not activate (or inactivate) any voltage dependent currents and that the recorded transients were indeed passive responses of the investigated cells. Three procedures were adopted to check the linearity of the voltage response.

1) After collecting 500 voltage transients evoked by a $-0.5\, \text{nA}$ current pulse, the same procedure was repeated with a current pulse of $-1.0\, \text{nA}$ (duration always $480\, \mu\text{s}$). The average transient then was scaled by a factor of 0.5 and superimposed on the average transient produced by the $-0.5\, \text{nA}$ current pulse. The two transients always superimposed within the 95% confidence band of the $-0.5\, \text{nA}$ current pulse response (Fig. 2A). The two recordings, however, were never pooled, and only the voltage transients to the $-0.5\, \text{nA}$ current pulse was used for analysis. The near perfect superposition of the two traces add additional support for the stationarity of the voltage transients over the time period required to acquire the transients for two different currents.

2) The voltage transient produced by the $-0.5\, \text{nA}$ current pulse was scaled by a factor of -1, plotted on a semilog scale and visually inspected for sag. In all cells used for analysis, the late linear part of the transient could be extended well into the baseline region where noise obscured the transient (Fig. 2B).

3) In 22 cells (of which 4 cells remained in the final group of 12 used in this paper), voltage-clamp experiments were performed either with two or one electrodes (15 cells with 2 electrodes, 7 cells with 1 electrode) to compile steady-state current-voltage relations. In all cases, the current-voltage relation was linear over the entire voltage range used and in most cases $\pm 10\, \text{mV}$ deviation from the holding potential at $-65\, \text{mV}$ (Fig. 2C).

Because none of the three tests could reveal any contribution of voltage activated conductances, we feel confident that the voltage transients used for final analysis were indeed passive responses of the cell’s membrane.

**HISTOLOGY AND MORPHOLOGICAL RECONSTRUCTION.** The electrophysiological measurements took between 20 and 30 min, during which time the Biocytin diffused into the cell. Before the electrodes were withdrawn from the cell, a video image was taken of the cell body and proximal dendrites. Therewith, the slice was removed from the recording chamber and placed in a fixative (0.1 M phosphate buffer containing 4% paraformaldehyde and 4% sucrose). The slices were stored in this fixative in the refrigerator for $\approx 8$ wk. They then were sent by express mail to Bern for histological processing and reconstruction.

The Biocytin-filled cells were visualized with the Vectastain ABC Kit (Vector Laboratories, Burlingame, CA) following a procedure first described by Horikawa and Armstrong (1988) and modified by O’Carroll et al. (1992) to include clearing and mounting of the tissue in glycerol. The slices were cleared with 50% glycerol in 0.1 M phosphate buffer for 1-3 h, followed by further clearing in 100% glycerol. The slices became transparent and were mounted on microscope slides in 100% glycerol and covered with a cover slip. The cells then were reconstructed without further delay ($<24$ h). Reconstruction was made by means of a computer-assisted reconstruction system (Eutectic 3D NTS, Eutectic Electronics, Raleigh, NC). Each cell was represented by $\approx 1,340$ data points in three-dimensional (3-D) space. The dendrites were assumed to be composed of short cylindrical segments defined by two successive data points and a diameter equal to the mean of the diameters measured at these two points. The total dendritic membrane surface area ($A_D$) was the sum of the surface areas of these cylindrical segments. Because it was not possible to focus through the densely stained cell body for measuring accurately the thickness of the soma, the membrane surface area of the soma ($A_S$) was approximated.
by a surface of a prolate spheroid formed by rotating an ellipse
about its major axis

\[ A_s = 2\pi b^2 + \frac{2\pi ab}{\epsilon} \arcsin (\epsilon) \]

where 2a and 2b denote the length of the major and minor axis,
respectively, of the ellipse representing the soma outline and \( \epsilon = \left( a^2 + b^2 \right)^{1/2}/a \).

The axon was not included in the morphological representation,
as simulations showed that its inclusion with the same membrane
properties as the rest of the neuron made a negligible difference
to the values obtained for \( R_m, R_s \), and \( C_m \). A comparison of the
dimensions of the soma and the proximal dendrites in the video
image taken during the experiments with the corresponding dimen-
sions after the staining procedure showed no evidence for tissue
shrinkage. This is consistent with observations on tissue shrinkage
made by other researchers using the same histological procedures
(Thurbon et al. 1994; Ulrich et al. 1994). Nominal resolution of
the reconstruction system was \( \approx 0.2 \) \( \mu m \).

The pattern of change in dendritic diameters at branch points
was quantified in terms of Rall’s ratio \( [\Sigma (D_{daughter}^{3/2})/D_{parent}^{3/2}] \).

**Modeling methods**

Combining physiological and morphological information of a
neuron into a compartmental model of that cell has been described
in detail by a number of researchers (Clements and Redman 1989;
Major et al. 1994; Rapp et al. 1994; Segev et al. 1989; Thurbon
et al. 1994; Ulrich et al. 1994). The common goal is to build a
passive electrical model of the neuron and find its values of \( R_i, R_m, \) and \( C_m \). This is achieved by closely matching the voltage
response of the model for a given current pulse at the soma to the
experimental voltage transient generated by the same current pulse
(assuming uniform passive membrane properties).

**MODELING TOOLS.** The simulator NEURON (Hines 1993) was
used running under Windows95 on a 90-MHz Pentium-based com-
puter. An integration time step of 25 \( \mu s \) was used. The fitting
algorithm used to match the model’s responses to the experimental
target transients is supplied by NEURON as a tool and is based
on minimizing a least square error function. The algorithm uses a
principal axis method using conjugate gradients (Brent 1973).
The cell’s morphology coded in a Eutectic file format was translated
by CABLE into a file that could be used directly as an input file
to NEURON specifying the 3-D morphology of the reconstructed
cells. Each dendritic section was subdivided into segments of
\( \approx 10 \) \( \mu m \).

**ONSET AND DURATION OF FIT INTERVAL.** The early part of the
voltage transient is most sensitive to the value of \( R_i \) (Fig. 3B),
whereas the later part of the transient is most sensitive to the value
of \( R_m \) (Fig. 3C) and a possible soma shunt conductance (Fig. 3D).
The amplitude and shape of both the late and early part of the
transient is important for extracting \( C_m \) (Fig. 3A). The selection
of the onset and end of the fit interval is therefore very crucial for
the validity of the parameter values. We chose as the duration of
the fit interval \( 2 \cdot \tau_m \). If a much longer fit interval is chosen (e.g.,
\( 3 \cdot \tau_m \)), too much weight is placed on fitting the late part of the
transient while neglecting the short early part of the target response
with a concomitant error in the estimated parameter values. Long
fit intervals require long simulation times as well. More crucial
than the length of the fit interval is, however, the selection of its
onset time. If the voltage transient is recorded with the current
injecting electrode (Major et al. 1994; Rapp et al. 1994; Thurbon
et al. 1994; Ulrich et al. 1994), the early part of the transient

![Fig. 3. Influence of the values of \( R_m, C_m, R_i \), and a somatic shunt conductance \((g-sh)\) on the shape of the voltage transient. Voltage response of a model \((C2-2201)\) to a current pulse \((480 \mu s, -0.5 nA)\) applied to the soma. A: influence of different \( C_m \) on the shape of the voltage transient \((R_m = 5 \, k\Omega/cm^2, R_i = 100 \, \Omega/cm)\). Early as well as the late part of the transient is affected. B: influence of different \( R_i \) on the shape of the voltage transient \((R_m = 5 \, k\Omega/cm^2, C_m = 1.5 \, \mu F/cm^2)\). Only the very early part of the transient is affected. C: influence of different \( R_m \) on the shape of the voltage transient \((R_i = 5 \, k\Omega/cm^2, C_m = 1.5 \, \mu F/cm^2)\). Only the late part of the transient is affected. D: influence of different somatic shunt conductances on the shape of the voltage transient \((R_m = 10 \, k\Omega/cm^2, R_i = 200 \, \Omega/cm, C_m = 1.5 \, \mu F/cm^2)\). Early part of the transient is not affected.](http://jn.physiology.org/Downloaded from http://jn.physiology.org)
FIG. 4. A comparison of the voltage transients recorded simultaneously via the current injecting electrode (-----) and via the 2nd, voltage-recording electrode (-----). Current-on and -off artifacts are clipped. Two curves only superimpose after ~20 ms from the end of the current pulse. B: same recording as in A but with expanded time scale. Arrow O_F indicates onset of fit interval. C: comparison of experimental voltage transient (-----) with model response (-----) to the same current pulse at the completion of the fitting procedure. O_C, onset of current pulse, O_F, onset of fit interval. Experimental transient rises more slowly than the model transient and is delayed by ~200 µs due to the fact that the current first has to charge the electrode capacitance and thus is no longer a rectangular pulse when charging the membrane capacitance.

is contaminated heavily with the electrode artifact, even if the capacitance neutralization is adjusted optimally. Major et al. (1994) have made a very careful analysis of the pipette artifacts and have chosen as the onset of the fit interval 2.5 ms after the end of the current pulse. Using a second electrode for passing the current minimizes the voltage artifact on the recording electrode. Because the geometric arrangement between the two electrodes was designed to minimize cross-capacitance (180° separation in the horizontal plane, 120° separation in the vertical plane, and an interposed grounded objective lens between the electrodes), the artifact caused by coupling capacitance was negligible. Even with well compensated electrodes (both capacity and bridge), the two simultaneously recorded voltage transients could deviate for more than 20 ms after the end of the current pulse (Fig. 4A). In this case, even a delay of 2.5 ms after the onset of the current pulse could not avoid artifact contamination in the early part of the target transient (Fig. 4B). Using the voltage transient recorded with the second electrode as target response circumvents this problem. However, there is still a remaining artifact caused by the residual uncompensated capacitance of the current electrode, which must be charged by the current pulse in addition to the membrane capacitance. Thus the current actually supplied to the neuron is not a rectangular pulse but a filtered pulse that is maintained for an unknown duration following its nominal end. Figure 4C compares the experimental voltage transient with the model response. It shows that the experimental transient is delayed by ~200 µs in comparison to the model response (which uses a rectangular current pulse). We chose as the onset of the fit interval 2.5 ms after the beginning of the current pulse to avoid this artifact. This selection is somewhat arbitrary, but it prevents us from overestimating \( R_i \). The same onset of the fit interval was applied if the voltage transient recorded with the current injecting electrode was used as the target response.

FIT ACCEPTANCE AND PRECISION OF PARAMETER ESTIMATION. In a first run, fits were accepted if they lay within the 95% confidence band around the average time course of the experimental target transient (Major et al. 1994). Accepted fits were further analyzed in terms of the residuals, defined as the difference between experimental transients and the fitted model response.

The calibration of the entire data acquisition, analysis and modeling chain (i.e., amplifiers, A/D-converter, modeling, and fitting software) was checked carefully using a resistor-capacitor network connected to the head stage of the Axoclamp-2B amplifiers and a corresponding computer model. The recovered values for the resistance and capacitance were well within the tolerance of the components used (1% resistor, 5% capacitor).

The performance of the fit algorithm was tested extensively.

### Table 1. Parameter identification is independent of start values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>“True” Value</th>
<th>Noise Free</th>
<th>Noise * 1</th>
<th>Noise * 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 10,000 \times R_m ), ( \Omega/cm^2 )</td>
<td>1</td>
<td>1.00008</td>
<td>1.00007</td>
<td>1.00072</td>
</tr>
<tr>
<td>( 100 \times R_i ), ( \Omega/cm )</td>
<td>2</td>
<td>1.9965</td>
<td>1.9948</td>
<td>1.9473</td>
</tr>
<tr>
<td>( C_n ), ( \mu F/cm^2 )</td>
<td>1.5</td>
<td>1.4997</td>
<td>1.4994</td>
<td>1.4937</td>
</tr>
</tbody>
</table>

Cell C2-1211-96 was used for the model. A noise-free transient was generated with known parameters for \( R_m \), \( R_i \), and \( C_n \). The parameters then were estimated by directly fitting the model’s response to this noise-free target response. In a second run experimental, noise was added to the noise-free transient and was used as the target response. The parameter search was repeated for increased noise contribution (factor 10). Each search was repeated four times with widely different initial values for \( 10,000 \times R_m \), \( 100 \times R_i \), and \( C_n \). For each noise level, the search always converged to exactly the same numbers independent of the start values.
TABLE 2. Influence of noise on the estimated parameter values

<table>
<thead>
<tr>
<th>Parameters</th>
<th>True Values</th>
<th>Start Values</th>
<th>End Values</th>
<th>End Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10,000 \times R_s$, $\Omega/cm^2$</td>
<td>1</td>
<td>2</td>
<td>1.0008</td>
<td>0.9984</td>
</tr>
<tr>
<td>$100 \times R_i$, $\mu F/cm^2$</td>
<td>2</td>
<td>1</td>
<td>1.9473</td>
<td>2.0534</td>
</tr>
<tr>
<td>$C_m$, $\mu F/cm^2$</td>
<td>1.5</td>
<td>1</td>
<td>1.4937</td>
<td>1.5095</td>
</tr>
</tbody>
</table>

Cell C2-1211-96 was used for the model. A noise-free transient was generated with known parameters for $R_s$, $R_i$, and $C_m$. Experimental noise multiplied times 10 was added to the noise-free transient and was used as the target response. The parameters then were estimated by directly fitting the model’s response to this target response. For each of the three different parameter searches, new noise was generated by shuffling 5- to 10-ms-long pieces of the baseline noise, to give the three sets of "end values."

Values for $R_s$, $R_i$, and $C_m$ were assumed and incorporated into compartmental models based on reconstructed neurons. A voltage transient then was generated by injecting a brief (480 $\mu s$) current pulse (amplitude $-0.5 \text{nA}$) into the soma and thereafter was used as the target function for the fit algorithm when the parameter search was started from many different initial values ($\pm 100\%$ difference from the true values). The parameter search always converged to precisely the same values which very closely corresponded to the true values, independent of the initial values (Table 1). This high precision in estimating the parameters from a noise-free transient might not be expected from an experimental, inherently noisy transient. To test the influence of the noise on the precision of the parameter search, noise-free transients generated from models with assumed values for $R_s$, $R_i$, and $C_m$ were generated. Fifty sweeps of experimental baseline noise were averaged and added to the noise-free transient. This “noisy” transient, produced by a model with known $R_s$, $R_i$, and $C_m$ then was used as the target function for estimating the above parameters. Again, independent of the starting values, the true parameters were recovered with a very high precision (Table 1). Even when the noise amplitude was multiplied by a factor of 10 (simulating a worst case scenario), the “true” values of the parameters of the model could still be recovered, and the algorithm always converged to exactly the same numbers (Table 1). With this procedure, we could never detect a local minimum in the parameter search. Five to 10-ms-long pieces of the noise then were shuffled randomly and the fitting procedure repeated with the same initial parameter values. The differences in the recovered values of the three parameters were very small, even in the case where the experimental noise amplitude was multiplied by a factor of 10 (Table 2).

Two such experiments are illustrated in Fig. 5. Cell C2-1211 was used for constructing the model. The true parameter values and the corresponding values of the estimated parameters are listed in Table 1. Figure 5A1 uses as a target response the model’s response with added experimental noise. In Fig. 5B1, the noise amplitude is multiplied by a factor of 10. In both cases, the fit superimposes the artificial target perfectly, and the responses cannot be distinguished. The corresponding residuals are plotted in Fig. 5, A2 and B2, respectively. The residuals are distributed equally around the fit. This analysis suggests that the estimated parameter values from a noisy experimental target transient are correct within a very narrow range around the true values, if stationarity in the target response can be ensured and if the assumption of homogeneous passive cable properties incorporated into the model is indeed correct. This procedure for testing the accuracy of the fitting algorithm does not solve the problem of possible nonuniqueness of the solutions because different sets of parameter values may fit the transient equally well within the boundaries given by the 95% confidence band (Rapp et al. 1994; Stratford et al. 1989). This problem becomes very obvious when additional parameters are introduced to the model, such as a shunt conductance at the soma. In these cases, it has been shown (see RESULTS, Fig. 10) that the residuals are not equally distributed around the model’s transient. If the assumptions of the model do not correspond to reality (e.g., nonhomogeneous passive membrane properties exist), a distinct pattern in the distribution of the residuals can be expected. This extensive analysis of the performance of the fitting algorithm first suggests that high-frequency noise in the voltage transients does not degrade significantly the values of the estimated parameters, second that the parameters can be estimated with very high accuracy, and third that inspection of the residuals (see Fig. 10) may provide additional information about the correct-
FIG. 6. Morphology, cell location within spinal cord and reconstruction of cell C2–1211, a presumed motoneuron. A: photomicrograph of the cell stained with Biocytin. B: location of the stained cell within the spinal cord slice. C: reconstructed cell body and proximal dendrites with the numbering of the proximal dendrites and axon. Axon could not be identified. D: reconstructed cell in the frontal plane. E: reconstructed cell in a sagittal view (---, upper and lower surface of the slice). F: dendrogram of the cell illustrated.

ness of the assumptions underlying the model if, and only if, the stationarity of the recorded voltage transient over time can be guaranteed (see later in the RESULTS). These factors, together with the fact that the confidence band was always very narrow, and many of the “optimal” fits just grazed the upper or lower limits of the confidence band (always in the very early part of the transient; see RESULTS section), led us to abstain from exploring the parameter space systematically to find upper and lower limits on the estimated parameters. We believe that the model parameters obtained from the “optimal” fit are very close to the true model values compatible with an adequate description of the experimental voltage transients.

**Determination of $L_{avg}$**

The electrotonic length $L_{OP}$ of each dendritic path of length $x_i$ is given by

$$L_{OP} = \sum_{i=1}^{n} \frac{\Delta x_i}{\lambda_i}$$

where

$$\lambda_i = \sqrt{\frac{a_i \cdot R_m}{2R_i}}$$
Δxᵢ is the length of the ith compartment such that xᵢ = NΔx, and

RESULTS

Morphology

Twelve cells were selected for morphological analysis from a total of 39 cells. The selection criterion was an apparently complete staining of the distal dendrites. Figures 6 and 7 show examples of one presumed motoneuron and one interneuron, respectively. All cells were located in the ventral half of the spinal cord slice. During the experiment, tentative identification of motoneurons was based on the size of the cell body. After staining and reconstruction, additional identification criteria were the precise location of the cell body and axon trajectories. We found large cells that sent their axons to the contralateral side, indicating that they were indeed interneurons. Size and location of cell body is not sufficient for positive identification of motoneurons. The large neuron illustrated in Fig. 6 was located in the motor nucleus, extending its dendrites over a large area of the ventral horn. Although its axon could not be identified with certainty, it was classified tentatively as a motoneuron. The large neuron depicted in Fig. 7 was located closer to the

**FIG. 7.** Morphology, cell location within spinal cord and reconstruction of cell C1–1411, a presumed interneuron. A: photomicrograph of the cell stained with Biocytin. B: location of the stained cell within the spinal cord slice. Note the axon trajectories extending toward the ventral horn of the spinal cord slice. C: reconstructed cell body and proximal dendrites with the numbering of the proximal dendrites and axon. Neurite no. 1 corresponds to the axon. D: reconstructed cell in the frontal plane. E: reconstructed cell in a sagittal view (—, upper and lower surface of the slice). F: dendrogram of cell illustrated.
membrane surface area (Chen and Wolpaw 1994). Selecting cell trees of rat lumbar spinal neurons extends almost radially to cortical pyramidal neurons or Purkinje cells, the dendritic substance portion of the dendritic tree was severed during with the degree of frequency adaptation; i.e., cell type could achieve all the dendrites had a smooth surface without spines scaling, and the absence of sag in the experimental voltage response to a brief current pulse applied to the soma could sustain high frequencies up to 350 Hz. The spike frequency at the beginning of the train increased linearly with increasing current. No secondary range could be observed. Frequency adaptation over the period of current injection (250 ms) ranged from almost no adaptation to almost complete adaptation. After morphological reconstruction, we could not associate putative motoneurons or interneurons with the degree of frequency adaptation; i.e., cell type could not be determined on the basis of frequency adaptation.

### Compartmental model

Compartmental modeling was used to estimate values of \( R_m \), \( R_s \), and \( C_m \) by directly fitting the model’s voltage response to the experimentally obtained target response. This exercise depends on several assumptions. Stationarity, linear scaling, and the absence of sag in the experimental voltage response to a brief current pulse applied to the soma could all be tested for adequately (see METHODS). However, the additional assumption of homogeneous membrane properties could not be tested for adequately (see METHODS). However, the additional assumption of homogeneous membrane properties

Table 3.

**Morphological data**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Fit</th>
<th>( A_S )</th>
<th>( A_D )</th>
<th>n-Stem</th>
<th>d-Stem</th>
<th>n-End</th>
<th>mbo</th>
<th>( d^{1/2} )</th>
<th>l-Tot</th>
<th>Major, ( \mu )m</th>
<th>Minor, ( \mu )m</th>
<th>( A_D/A_S )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3-1111</td>
<td>MN</td>
<td>yes</td>
<td>1387</td>
<td>3052</td>
<td>8</td>
<td>3.1</td>
<td>10</td>
<td>3</td>
<td>0.86</td>
<td>856</td>
<td>38.14</td>
<td>13.24</td>
</tr>
<tr>
<td>C2-1211</td>
<td>MN</td>
<td>yes</td>
<td>788</td>
<td>6597</td>
<td>3</td>
<td>5</td>
<td>14</td>
<td>7</td>
<td>1.1</td>
<td>2270</td>
<td>22.13</td>
<td>13.84</td>
</tr>
<tr>
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<td>MN</td>
<td>yes</td>
<td>1128</td>
<td>6887</td>
<td>7</td>
<td>3.3</td>
<td>16</td>
<td>5</td>
<td>0.84</td>
<td>3506</td>
<td>23.17</td>
<td>17.62</td>
</tr>
<tr>
<td>C3-1111</td>
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<td>yes</td>
<td>1936</td>
<td>5597</td>
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<td>3.4</td>
<td>11</td>
<td>3</td>
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<td>2449</td>
<td>33.11</td>
<td>21.34</td>
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<tr>
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<td>yes</td>
<td>2230</td>
<td>10431</td>
<td>6</td>
<td>4.2</td>
<td>38</td>
<td>6</td>
<td>0.96</td>
<td>5905</td>
<td>38.92</td>
<td>21.11</td>
</tr>
<tr>
<td>C1-2101</td>
<td>MN</td>
<td>yes</td>
<td>2030</td>
<td>10481</td>
<td>6</td>
<td>4.7</td>
<td>21</td>
<td>5</td>
<td>0.96</td>
<td>3265</td>
<td>31.11</td>
<td>22.77</td>
</tr>
<tr>
<td>C2-2101</td>
<td>IN</td>
<td>no</td>
<td>1724</td>
<td>11824</td>
<td>3</td>
<td>7.1</td>
<td>24</td>
<td>7</td>
<td>1.19</td>
<td>4545</td>
<td>32.62</td>
<td>19.25</td>
</tr>
<tr>
<td>C2-2201</td>
<td>IN</td>
<td>yes</td>
<td>1113</td>
<td>3595</td>
<td>4</td>
<td>2.3</td>
<td>9</td>
<td>3</td>
<td>0.79</td>
<td>1811</td>
<td>27.55</td>
<td>14.89</td>
</tr>
<tr>
<td>C4-2201</td>
<td>IN</td>
<td>yes</td>
<td>3177</td>
<td>8940</td>
<td>7</td>
<td>3.2</td>
<td>14</td>
<td>3</td>
<td>0.97</td>
<td>3770</td>
<td>36.11</td>
<td>29.71</td>
</tr>
<tr>
<td>C2-1002</td>
<td>IN</td>
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<td>2906</td>
<td>9067</td>
<td>5</td>
<td>5.9</td>
<td>9</td>
<td>3</td>
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<td>2809</td>
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<tr>
<td>C3-1002</td>
<td>MN</td>
<td>yes</td>
<td>3524</td>
<td>7544</td>
<td>6</td>
<td>5</td>
<td>18</td>
<td>5</td>
<td>0.95</td>
<td>2727</td>
<td>45.32</td>
<td>28.59</td>
</tr>
</tbody>
</table>

Mean values are ± SD. MN, presumed motoneuron; IN, presumed interneuron; Fit, gave satisfactory fits at the ~95% confidence level; \( A_S \), somatic membrane surface area (\( \mu \)m²); \( A_D \), dendritic membrane surface area (\( \mu \)m²); n-Stem, number of stem dendrites; d-Stem, mean dendritic stem diameter (\( \mu \)m); n-End, number of dendritic terminals; mbo, maximal branch order; \( d^{1/2} \), mean Rall’s ratio; l-Tot, total dendritic path length (\( \mu \)m); major and minor, axes of elliptical soma. Shaded rows indicate the cells which remained in the final analysis.

### Passive electrical parameters

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>( R_s, \text{M}\Omega )</th>
<th>( \tau_m, \text{ms} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3-1111</td>
<td>MN</td>
<td>162</td>
</tr>
<tr>
<td>C2-1211</td>
<td>MN</td>
<td>384*</td>
</tr>
<tr>
<td>C1-1311</td>
<td>MN</td>
<td>85</td>
</tr>
<tr>
<td>C3-1311</td>
<td>MN</td>
<td>64</td>
</tr>
<tr>
<td>C1-1411</td>
<td>IN</td>
<td>117*</td>
</tr>
<tr>
<td>C1-2101</td>
<td>MN</td>
<td>93</td>
</tr>
<tr>
<td>C2-2101</td>
<td>MN</td>
<td>n.a.</td>
</tr>
<tr>
<td>C3-2101</td>
<td>IN</td>
<td>64</td>
</tr>
<tr>
<td>C2-2201</td>
<td>IN</td>
<td>127</td>
</tr>
<tr>
<td>C4-2201</td>
<td>IN</td>
<td>67</td>
</tr>
<tr>
<td>C2-1002</td>
<td>IN</td>
<td>29</td>
</tr>
<tr>
<td>C3-1002</td>
<td>MN</td>
<td>104</td>
</tr>
</tbody>
</table>

Mean values are ± SD. \( R_s \), input resistance calculated from long hyperpolarizing current pulses, * slope resistance estimated from I-V curves.; \( \tau_m \), membrane time constant equated to system time constant (\( \tau_N \)).

---

**electrophysiological properties of the cells could not be used for positive identification as well (see further text).**

As can be seen from inspection of Figs. 6E and 7E, a substantial portion of the dendritic tree was severed during the slicing process. This cannot be avoided because, unlike cortical pyramidal neurons or Purkinje cells, the dendritic tree of rat lumbar spinal neurons extends almost radially from the cell body (Chen and Wolpaw 1994). Selecting cell bodies deep in the slice would keep more of the dendritic tree intact. Impaired visibility of the cell body, however, made such an approach impractical. Table 3 summarizes some morphological parameters of each cell analyzed. Because all the dendrites had a smooth surface without spines or swellings, the surface area of the neurons could be estimated accurately, although the measured diameter values of the distal dendrites were affected with considerable uncertainty caused by the limited resolution of the reconstruction system.

The mean ratio of \( A_D/A_S \) was 4.25 ± 2.01 (SD: range 2.1–8.4), indicating that the somatic surface area contributed significantly to the total membrane surface area of the cell. In the intact spinal cord, this ratio might be higher by a factor of ~2. The small ratio of \( A_D/A_S \) and the absence of spines makes these cells well suited for modeling and cable parameter identification.

**Electrophysiological measurements**

Electrophysiological data were obtained from 39 cells. The data from the 12 cells for which we obtained satisfactory morphological data are presented here. Table 4 indicates the input resistance and membrane time constant for each of these cells.

All cells could be made to fire repetitively. All of them could sustain high frequencies ~350 Hz. The spike frequency at the beginning of the train increased linearly with increasing current. No secondary range could be observed. Frequency adaptation over the period of current injection (250 ms) ranged from almost no adaptation to almost complete adaptation. After morphological reconstruction, we could not associate putative motoneurons or interneurons with the degree of frequency adaptation; i.e., cell type could not be determined on the basis of frequency adaptation.

**Compartmental model**

Compartmental modeling was used to estimate values of \( R_m \), \( R_s \), and \( C_m \) by directly fitting the model’s voltage response to the experimentally obtained target response. This exercise depends on several assumptions. Stationarity, linear scaling, and the absence of sag in the experimental voltage response to a brief current pulse applied to the soma could all be tested for adequately (see METHODS). However, the additional assumption of homogeneous membrane properties...
TABLE 5. Summary of the electrical parameters obtained from direct optimal fits between model and experimental responses

<table>
<thead>
<tr>
<th>Cell</th>
<th>Type</th>
<th>#-El.</th>
<th>$R_m$, kΩ/cm²</th>
<th>$R_i$, Ω/cm</th>
<th>$C_m$, μF/cm²</th>
<th>Fit Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3-1111</td>
<td>MN</td>
<td>1</td>
<td>8.79</td>
<td>152</td>
<td>2.11</td>
<td>2.5/35</td>
</tr>
<tr>
<td>C2-1211</td>
<td>MN</td>
<td>1</td>
<td>26.54</td>
<td>482</td>
<td>1.3</td>
<td>2.5/60</td>
</tr>
<tr>
<td>C1-1311</td>
<td>MN</td>
<td>1</td>
<td>6.28</td>
<td>338</td>
<td>2.3</td>
<td>2.5/30</td>
</tr>
<tr>
<td>C3-1311</td>
<td>MN</td>
<td>2</td>
<td>4.24</td>
<td>119</td>
<td>2.7</td>
<td>2.5/25</td>
</tr>
<tr>
<td>C1-1411</td>
<td>IN</td>
<td>1</td>
<td>10.88</td>
<td>167</td>
<td>3.1</td>
<td>2.5/46</td>
</tr>
<tr>
<td>C1-2101</td>
<td>MN</td>
<td>2</td>
<td>5.96</td>
<td>78</td>
<td>1.7</td>
<td>2.5/18</td>
</tr>
<tr>
<td>C2-2101</td>
<td>MN</td>
<td>2</td>
<td>8.68</td>
<td>217</td>
<td>2.0</td>
<td>2.5/28</td>
</tr>
<tr>
<td>C2-2201</td>
<td>IN</td>
<td>2</td>
<td>4.95</td>
<td>77</td>
<td>2.3</td>
<td>2.5/25</td>
</tr>
<tr>
<td>C4-2201</td>
<td>IN</td>
<td>2</td>
<td>6.20</td>
<td>72</td>
<td>2.7</td>
<td>2.5/30</td>
</tr>
<tr>
<td>C3-1002</td>
<td>MN</td>
<td>2</td>
<td>9.77</td>
<td>541</td>
<td>2.6</td>
<td>2.5/40</td>
</tr>
</tbody>
</table>

Mean 9.23 ± 6.45 224.30 ± 171.78 2.28 ± 0.54

Fits accepted by the ~95% confidence band test. Mean values are ±SD. #-El. One- to two-electrode experiment; $R_m$, membrane resistivity; $R_i$, cytosolic resistivity; $C_m$, membrane capacitance; Fit Interval: first number; onset of fit interval after beginning of current pulse (ms), second number; duration of fit interval (ms). The four cells highlighted passed the final test for model adequacy.

could not be tested experimentally. Therefore the possibility remains that the wrong models were fitted to the experiments with severe consequences on the validity of the estimated parameters. In an attempt to evaluate model adequacy, we have analyzed the residuals between optimal fit and experimental target responses and compared them with the residuals obtained by fitting known but inadequate models to transients generated by models with homogeneous membrane parameters.
properties. In a first run, fits were accepted if they did not escape the 95% confidence band (Major et al. 1994). Thereafter the shape of the residuals was inspected and the results classified accordingly.

**OPTIMAL FITS AND PARAMETER VALUES.** Of the 12 cells analyzed, 10 produced acceptable fits as judged by the confidence band test. Table 5 summarizes the values obtained for \( R_m \), \( R_i \), and \( C_m \).

The mean optimal \( C_m \) of 2.28 \( \mu \)F/cm\(^2\) is about three times higher than determined in a similar study on hippocampal pyramidal cells (Major et al. 1994). Mean \( R_i \) was 224 \( \Omega/cm \) and \( R_m \) was 9.23 k\( \Omega/cm^2 \). Of the three values, \( C_m \) is probably the most reliable because it is the most robust parameter in the fitting procedure. \( R_i \) is the least reliable parameter when using a single electrode because it is mostly determined by the initial part of the transient, the part that also is affected the most by electrode artifacts. Depending on the onset time of the fit interval, some of the artifact might have been included (if the interval started too early), or alternatively, valuable information was lost if the fit interval started too late. As long as no somatic shunts are allowed in the model, \( R_m \) is robust and not affected by electrode artifacts (see later text). Other possible sources of errors will be explored in **DISCUSSION**.

The results summarized in Table 5 could be divided into two groups as judged by the distribution of the residuals between the target response and the optimal fit. Three examples of optimal fits with evenly distributed residuals are shown in Fig. 8. The left column in Fig. 8 (A1, B1, and C1) illustrate that the experimentally obtained target response (fine line) and the model response (thick line) superimpose perfectly. The corresponding residuals between target response and model response are shown in the right column of Fig. 8 (A2, B2, and C2) together with the 95% confidence band. In 4 of the 10 cells, the residuals were distributed equally around the model response as shown for the three cells in Fig. 8. This even distribution of the residuals suggests that the three parameter, homogeneous model was a very good description of the real cells.

In 6 of 10 cells the residuals, albeit within the 95% confidence band, showed a characteristic time course. As an example, two cells are illustrated in Fig. 9. As we have shown (in **METHODS**) that the experimental voltage transient is stationary, superimposes linearly and is devoid of sag, thus avoiding spurious curvature in the average target response, the characteristic shape of the residuals suggests that the model used in the fitting procedure was not an adequate description of the neuron. Nonhomogeneous membrane properties are a likely cause of these residuals.

An obvious and most likely candidate for membrane inhomogeneity is a less than perfect seal at the electrode tip or a genuine low membrane resistivity of the soma. The use of two electrodes is more likely to introduce a somatic leak conductance than when one is used. Unfortunately, when a fourth parameter (\( g_{shunt} \) representing a somatic leak conductance) is introduced into the model as a free parameter, nonuniqueness in the solution becomes a very serious problem, as others have already noted (Major et al. 1994). We have performed an extensive analysis of the four-parameter model with artificial target responses from models with known parameters. Because variations in \( g_{shunt} \) and \( R_m \) lead to changes in the shape of the model voltage response that are virtually indistinguishable (see Fig. 3), almost any reciprocal pair of parameter values for \( R_m \) and \( g_{shunt} \) will result in

**FIG. 9.** Optimal fits of 2 cells with a distinct time course of the residuals. A1: cell C2–1211, a presumed motoneuron. B1: cell C2–2101, a presumed motoneuron. A2 and B2: corresponding residuals and 95% confidence band. Residuals are blanked between the onset of the current pulse and the onset of the fit interval. Residuals stay within the confidence band but display a characteristic time course.
acceptable fits. In principal, \( g_{\text{shunt}} \) can vary from zero up to the cell’s total input conductance and still yield acceptable fits. As \( g_{\text{shunt}} \) increases, \( R_m \) increases up to extreme, implausible values.

In the six cells, where the residuals were not evenly distributed, we allowed \( g_{\text{shunt}} \) to drift away from zero. A range of values for \( g_{\text{shunt}} \) between 0.1 and 6.3 nS was found. In all six cases, the mean squared error of the fit decreased as expected, but the time course of the residuals did not even out as in Fig. 8. We must conclude that we have no reliable way to prove whether a significant somatic shunt is present or not in these neurons. Although the introduction of \( g_{\text{shunt}} \) leads to a major uncertainty in the estimation of \( R_m \), the uncertainty in the estimated values for \( C_m \) and \( R_i \) is not large.

Because of these difficulties and uncertainties introduced by a fourth parameter, we abstained from further exploration of the contribution of a somatic shunt on the parameter values, and we present the results as obtained from the three parameter, homogeneous model. We must keep in mind, however, that if a somatic shunt were present in the six cells with a characteristic time course in the unevenly distributed residuals, \( C_m \) and \( R_i \) would have been overestimated slightly.

The introduction of any other additional parameters for taking into account membrane inhomogeneities would probably lead to similar nonuniqueness difficulties. With respect to these difficulties, a search for models that would have eliminated the characteristic time course in the residuals would probable have been a wasted effort. However, we
have attempted to identify the most likely model that, if used to fit the target transient, could explain the specific time course of the residuals.

Taking the morphology of cell C1-2101 as a model, artificial, noise-free transients were generated for known parameter values of $R_m$, $R_i$, and $C_m$ and their inhomogeneous distribution. The following inhomogeneities were considered: $R_m$-soma lower or higher than $R_m$-dendrite, $C_m$-soma lower or higher than in the dendrites, $R_i$ in the soma, and proximal dendrites lower or higher than in the distal dendrites. The six transients from these four-parameter models then were used to fit the voltage response of a three-parameter, homogeneous model. In all six cases, the fits were virtually indistinguishable from the target responses (within 95% confidence limits) but with characteristic distributions in the residuals (Fig. 10). The corresponding estimated values for $R_m$, $R_i$, and $C_m$ are listed in Table 6.

Unfortunately, the time course of the residuals resulting from fitting the “wrong” models to the target responses do not contain a “unique” signature that would allow unequivocal identification of the wrong model. Comparing the shape of the time courses of the residuals in Fig. 9 with the residuals in Fig. 10, however, suggests that the model with a higher somatic membrane resistivity (Fig. 10A2) leads to a distribution of the residuals compatible with the results illustrated in Fig. 9. Although this exercise might push the analysis too far, inspection of the estimated parameter values in Table 6 clearly reveals that, even in the case of an inadequate model, the retrieved parameter values are close to the values of the underlying homogeneous model. In addition, the values listed in Table 6 illustrate whether a particular parameter value has been over- or underestimated with regard to a particular inhomogeneous model.

The analysis presented above illustrates that $R_i$ is the most sensitive parameter to very large errors if one tries to fit the wrong model to the target response. In contrast, the estimated value of $C_m$ turned out to be the most robust and most reliable. This has an intuitive explanation in the fact that many dendrites were severed during the slicing process, leading to an underestimation of mean $C_m$ compared (early part only) (see Fig. 3). In previous studies, $C_m$ of 4 $\mu F/cm^2$ were reported by Barrett and Crill (1974). More recent investigations (Burke et al. 1994; Clements and Redman 1989) have pointed out that these high values of $C_m$ were the result of neglecting a somatic shunt conductance. The introduction of a somatic shunt lowered $C_m$ close to the generally accepted value of 1.0 $\mu F/cm^2$. To evaluate this possibility, we have fixed $C_m = 1.0 \mu F/cm^2$ and let $R_m$, $R_i$, and $g_{shunt}$ (soma) be free parameters. In all cases ($n = 4$), no acceptable fits could be obtained as evaluated by the 95% confidence test. In addition, $g_{shunt}$ always converged exactly to the value of the cell’s input conductance with a concomitant high value of $R_m (>1 G\Omega/cm^2)$. $R_i$ settled at impossible values of 5 $\Omega/cm$. We must conclude, that omitting a somatic shunt conductance does not explain the high value of $C_m$ found in our study.

It is possible that we have missed the most distal dendrites in the reconstruction. To evaluate the contribution of possibly missed distal dendrites to the estimated parameter values, we attached to all existing dendrites of a reconstructed cell 100-um-long terminal sections, with a diameter tapering from 0.5 to 1.0 $\mu m$. This increased the total dendritic path length by 17% and the total surface area by 5.3%. This substantial elongation of the distal dendrites changed the retrieved parameters only slightly. $C_m$ was lowered by <7%. Because we have no reason to suspect that we have missed such a large part of the dendritic tree in the reconstruction process, we conclude that an incomplete reconstruction would cause insignificant overestimate of $C_m$.

As a result of the extensive evaluation of the fitting procedure presented above, we conclude that the parameter values of the optimal fits obtained from the four cells in which the residuals were evenly distributed around the target transient (highlighted rows in Table 5), represent the most likely values for $R_m$, $R_i$, and $C_m$ (summarized in Table 7).

### Passive electrotonic structure

The mean electrotonic path length ($L_{avg}$) for the four cells remaining after the analysis detailed above was 0.69 ± 0.10 (range 0.84–0.63). Because of the slicing, many dendrites were cut a short distance from the soma. The electrotonic path length of individual dendrites was therefore very different, e.g., ranging from 0.016 to 1.25, in cell C3-1311. The fact that many dendrites were severed during the slicing process leads to an underestimation of mean $L_{avg}$ compared with $L_{avg}$ in the intact spinal cord. If only the uncut dendrites are taken into account, mean $L_{avg}$ would be 0.85 ± 0.14 (12 dendrites of 4 cells).

The input resistances as measured experimentally are

---

**TABLE 6. Estimated parameters obtained from fitting deliberately a wrong, homogeneous, 3-parameter model to target transients obtained from an inhomogeneous, 4-parameter model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>True Values</th>
<th>Homogeneous Model</th>
<th>$R_m$ Soma, k$\Omega$/cm$^2$</th>
<th>$C_m$ Soma, $\mu F/cm^2$</th>
<th>$R_i$ Proximal Dendrite and Soma, $\Omega/cm$</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_m$, k$\Omega$/cm$^2$</td>
<td>10</td>
<td>7.831</td>
<td>11.236</td>
<td>8.757</td>
<td>10.111</td>
<td>9.560 ± 1.21</td>
</tr>
<tr>
<td>$R_i$, $\Omega/cm$</td>
<td>200</td>
<td>302</td>
<td>169</td>
<td>565</td>
<td>112</td>
<td>251.3 ± 168.60</td>
</tr>
<tr>
<td>$C_m$, $\mu F/cm^2$</td>
<td>1.5</td>
<td>1.68</td>
<td>1.43</td>
<td>1.76</td>
<td>1.41</td>
<td>1.540 ± 0.15</td>
</tr>
</tbody>
</table>

Mean values are ± SD. The target transient was generated from a model with inhomogeneous membrane properties (4-parameter model). The parameter values deviated from the homogeneous model by the properties listed in the six columns (inhomogeneous model). The parameters $R_m$, $R_i$, and $C_m$ were then estimated assuming a homogeneous, 3-parameter model.
TABLE 7. Electrical parameters obtained from compartmental computer modeling and comparison with the experimental results for the four cells finally accepted

<table>
<thead>
<tr>
<th>Cell</th>
<th>Type</th>
<th>$R_m$, kΩ/cm$^2$</th>
<th>$R_i$, Ω/cm</th>
<th>$C_m$, μF/cm$^2$</th>
<th>Model, ms</th>
<th>Experiment, ms</th>
<th>Model, MΩ</th>
<th>Experiment, MΩ</th>
<th>$L_{avg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3-1311</td>
<td>MN</td>
<td>4.24</td>
<td>119</td>
<td>2.7</td>
<td>11.7</td>
<td>11.7</td>
<td>70</td>
<td>64</td>
<td>1.18</td>
</tr>
<tr>
<td>C1-2101</td>
<td>MN</td>
<td>5.96</td>
<td>78</td>
<td>1.7</td>
<td>10.1</td>
<td>9.7</td>
<td>92</td>
<td>93</td>
<td>0.61</td>
</tr>
<tr>
<td>C2-2201</td>
<td>IN</td>
<td>4.95</td>
<td>77</td>
<td>2.3</td>
<td>11.4</td>
<td>11.9</td>
<td>107</td>
<td>127</td>
<td>0.68</td>
</tr>
<tr>
<td>C4-2201</td>
<td>IN</td>
<td>6.20</td>
<td>72</td>
<td>2.7</td>
<td>16.7</td>
<td>15.9</td>
<td>67</td>
<td>67</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Mean values are ±SD. $R_m$, membrane resistivity; $R_i$, cytosolic resistivity; $C_m$, membrane capacitance; $\tau_m$, membrane time constants; $R_N$, steady-state input resistance; $L_{avg}$, mean electronic dendritic path length.

Discussion

The search for the passive membrane properties and cytoplasmatic resistivity of motoneurons has a long history (Rall et al. 1992). Technical innovations in electrophysiological measurements or morphological reconstructions have provided the impetus to improve on the inadequacies of previous measurements and this investigation is no exception. Previous measurements of the cable properties of motoneurons have had to contend with distortions to the analysis caused by electrode artifacts and tissue shrinkage. In this study, we have eliminated the latter and minimized the former by using an in vitro slice preparation and whole cell recording with two electrodes: one for voltage and the other for current. In each of four neurons, the voltage response to a brief current pulse could be matched very precisely to the transient generated by a compartmental model for that neuron, based on the three parameters $R_m$, $R_i$, and $C_m$. Each of these parameters was most sensitive to the voltage response over different time periods, providing an important element of uniqueness in their determination. In six other neurons for which high-quality morphological reconstruction and electrophysiology was obtained, the same precision in matching the model transient to the neurons responses could not be achieved. The mean (±SD) values for $R_m$ and $R_i$ for these neurons ($11.8 ± 6.7$ kΩ/cm$^2$ and $316 ± 151$ Ω/cm, respectively; $n = 6$) were not significantly different from the corresponding values for the four precisely modeled neurons ($5.3 ± 0.9$ kΩ/cm$^2$ and $87 ± 22$ Ω/cm; $n = 4$), using a modified $t$-test for unequal variances (Snedecor and Cochran 1967). Similarly, the difference in the membrane capacitance was not significant ($2.2 ± 0.6$ μF/cm$^2$, $n = 6$ cf. $2.4 ± 0.4$ μF/cm$^2$, $n = 4$) for the two data sets.

Identification of motoneurons and interneurons

It became clear that large neurons in the ventral horn could not be reliably identified as motoneurons, as the axons of some of these neurons crossed to the contralateral side. These neurons must be interneurons. Only rarely could the axon be followed to the ventral root exit region, and the neurons of these axons were classified as motoneurons. When the axon trajectory could not be followed, cell identification was not reliable. As there appeared to be no systematic difference between the cable parameters for putative motoneurons and interneurons, the results for both neuron types have been combined.

Passive cable parameters

CYTOPLASMIC RESISTIVITY ($R_c = 87 ± 22$ Ω/cm). The lower limit of this parameter is 43 Ωcm, based on the resistivity of a solution combining 50 mM NaCl and 110 mM KCl in 1% agar at 37°C (Clements and Redman 1989). As the chloride concentration in the cytoplasm is low, electrical neutrality is preserved by large negatively charged macromolecules. These less mobile charge carriers, and the presence of organelles, cytoskeletal elements, and charge binding, should result in $R_i$ being well in excess of this value. The only reported direct measure of $R_i$ is $70 ± 15$ Ω/cm in mammalian motoneurons (Barrett and Crill 1974). More recently, investigations using combined morphological reconstruction and single electrode recording from a variety of neuron types have reported $R_i$ values in the range of 70–520 Ω/cm (Clements and Redman 1989; Major et al. 1994; Shelton 1985; Thurbon et al. 1994; Ulrich et al. 1994). A more powerful method based on whole cell recording at the soma and the apical dendrite of a cortical pyramid neuron, combined with morphological reconstruction has yielded values of $R_i$ between 70 and 100 Ω/cm (Stuart and Spruston 1998). $R_i$ may vary for different types of neuron if the proportion of nonconducting elements in the cytoplasm varies. It also may be reduced by dialysis, as the large cytoplasmic molecules will be diluted by the electrode solution.

The estimate for $R_i$ is most sensitive to the initial part of the voltage decay (Fig. 3B), and it is this region of the response that is most affected by capacitive artifact. Measurements using a single-electrode technique are more affected by this artifact than are two-electrode measurements, as charging and discharging the capacitance of the current compared with the input resistances of the models and listed in Table 7. In the same table, the experimentally obtained membrane time constants are compared with the system time constants of the model with the corresponding parameters of $R_m$, $R_i$, and $C_m$. There is a very close correspondence between the experimental and modeling results. This strengthens our conclusion, that the final mean values estimated for the parameters $R_m = 5.34 ± 0.91$ (SD) kΩ/cm$^2$, $R_i = 86.5 ± 21.83$ Ω/cm, and $C_m = 2.35 ± 0.47$ μF/cm$^2$ provide an adequate description of the cable properties of the four cells listed in Table 7.
electrode distorts the voltage transient at early times. When two electrodes are used, provided there is negligible coupling capacitance between them, the only distortion remaining arises from low-pass filtering of the current pulse by the capacitance of the current passing electrode. This results in a nonrectangular current pulse entering the neuron, and it will cause an error in the calculation for $R_i$. Its effect is minimized by delaying the time at which the voltage transient is modeled. None of the single electrode measurements in this study (Table 5) resulted in ideal fits to the model, and all of them resulted in higher values of $R_i$ than those obtained for the four ideal results.

**MEMBRANE RESISTIVITY ($R_m = 5.3 \pm 0.9 \, \text{k} \Omega/\text{cm}^2$).** Reliable determination of this parameter represents a major challenge, even with the improved techniques used in this investigation. The main difficulty is that the presence of a leak conductance at the soma can give an artifactually low value of $R_m$ if the leak conductance is ignored (Fig. 3, C and D). When both $R_m$ and somatic shunt parameters are introduced to the compartmental model, equally good fits are obtained for a large range of shunt conductance and an inversely large range of $R_m$. Although the electrode seal resistance is known before the somatic membrane is ruptured, changes to this seal resistance may occur after rupturing the patch under the first electrode. Any leak conductance caused by rupturing the patch under the second electrode will be detected in the holding current of the first electrode, and the experiment discontinued if this occurs. Also, the holding current must not increase during the recording. Even when these requirements are met, there is no secure way of knowing the leak resistance associated with the first electrode. For all the calculations documented in Table 5, the leak conductance was assumed to be zero, and this may cause an underestimate of $R_m$.

Values of $R_m$ reported for other neurons when caesium was not present to block the resting potassium conductance range from 7 to 220 $\text{k} \Omega/\text{cm}^2$ (Major et al. 1994; Thurbon et al. 1994). When caesium was present in the electrode solution, Ulrich et al. (1994) reported a value of $R_m = 17.5 \, \text{k} \Omega/\text{cm}^2$ for cultured motoneurons. As for $R_m$, different neuron types may have very different resting conductances and hence values of $R_m$.

**MEMBRANE CAPACITANCE ($C_m = 2.4 \pm 0.5 \, \mu\text{F/cm}^2$).** This parameter was the most robust of the three in the fitting procedure. Major departures from uniform membrane resistance did not alter $C_m$ very much from its true value (Table 6). Thus the only potential source of significant error would be in the determination of membrane surface area that trades off with the estimated value of $C_m$. A linear scaling of each compartment by a factor of 1.5 for both length and diameter would reduce $C_m$ by a factor of 2.25, with a concomitant increase of $R_m$ by 50% (following the method given in Major et al. 1994). Such a correction of the morphology would lead to a value of $C_m$ close to the commonly assumed value of 1.0 $\mu\text{F/cm}^2$ (to conserve $\tau_m$, $R_m$ must change correspondingly). The calculation of the surface area is relatively simple for motoneurons. They do not have spines, which can make surface area calculations very difficult, and their dendrites branch infrequently. Electron microscopy of soma and dendrites of motoneurons indicates negligible undulation and no infolding of the surface membrane (Conradi 1969). The histological procedure did not cause shrinkage, as video images of the soma and proximal dendrites taken at the beginning and end of the experiment provided an independent check on cell dimensions, as well as any possible swelling of the cell during the experiment. Therefore we consider an underestimation of the total surface area by a factor of 2.25 as most unlikely.

The value obtained for $C_m$ is very different from 1 $\mu\text{F/cm}^2$, commonly assumed for neuronal membrane, and from the values obtained for other neuron types by similar fitting procedures to those used in this experiment [1.1 ± 0.2 $\mu\text{F/cm}^2$ for cultured motoneurons (Ulrich et al. 1994); 0.7 ± 0.8 $\mu\text{F/cm}^2$ for CA3 pyramidal cells (Major et al. 1994)]. Other investigators using combined morphology and electrophysiological measurements on motoneurons have reported a wide range of values for $C_m$ [2 $\mu\text{F/cm}^2$ (Lux et al. 1970); 2-4 $\mu\text{F/cm}^2$ (Barrett and Crill 1974); 3 $\mu\text{F/cm}^2$ (Gibb 1959); 2 $\mu\text{F/cm}^2$ (Major et al. 1994); Thurbon et al. 1994]).

![FIG. 11. Electrotonic transforms for cell C1–2101, a presumed motoneuron.](image)

A: line drawing of the reconstructed neuron. B: electrotonic profile for the cell depicted in A for a DC potential applied to different sites on the dendrites as it decays toward the soma. C: same as in B but for the application of a 50-Hz sine wave signal instead of a DC signal. D: electrotonic profile of the cell in A for a DC signal applied to the soma as it decays to different sites on the dendrites. E: same as in D but for a 50-Hz sine wave. Scale bar is the distance over which the voltage attenuation is 1/e. Note different scale bars for the top and bottom pair of the cells.
1.8 μF/cm² (Nitzan et al. 1990)]. The capacitance of a 5.7-nm–thick membrane with a lipid/protein mix depends critically on the proportion of protein present. Assuming a 50/50 composition (Alberts et al. 1989; Ch. 6), a dielectric constant for protein of 30 (Smith et al. 1993) and for lipid of 2 (Fettiplace et al. 1971), the membrane capacitance would be 2.3 μF/cm². If the protein component differs in different cells or in different regions of a cell, cell-to-cell variability and nonuniform distributions in $C_m$ might be expected.

Electrotonic profile of motoneurons

In Fig. 11A, C1–2101 is drawn in real length units (micrometers). The same cell is redrawn with four different length scalings in Fig. 11, B–E. The relative scales correspond to the electrotonic characteristics of the dendrites for current spreading from the dendrites toward the soma (Fig. 11B for steady-state current and Fig. 11C for 50 Hz) and for current spreading distally from the soma (Fig. 11D for steady-state current and Fig. 11E for 50 Hz). The scale bar corresponds to the distance over which the attenuation is $e^{-1}$. This electrotonic transformation is described in Zador et al. (1995). Figure 11 illustrates dramatically how a neuron may be relatively compact electrotonically for DC voltages applied to the soma but heavily attenuates AC currents spreading centrally from the dendrites. As fast synaptic potentials at their dendritic site of generation can peak in ~0.5 ms, and effectively terminate in ~10 ms, their frequency spectrum ranges from 25 to 500 Hz. Figure 11 indicates that while large regions of the dendritic tree will be reasonably well clamped for DC potentials, this will not be true for fast voltage transients generated in the dendrites. The rapid decay of synaptic potentials as they spread from dendrites to soma is illustrated later in a more conventional way.

Simulation of synaptic input

Synaptic input was simulated as a conductance change in the form of an $\alpha$ function with a time to peak of 0.3 ms. In Fig. 12A, an excitatory postsynaptic potential (EPSP) is plotted as recorded from the dendritic site d1, where the conductance change was applied, together with the same EPSP as it reaches the soma. As suggested by the electrotonic transform in Fig. 11, the EPSP decrements very rapidly as it spreads toward the soma. In Fig. 12B, three EPSPs recorded from the soma are shown. They were elicited by the same conductance change either applied to the soma (s), to the dendritic location (d1), or to the distal dendritic location d2. In Fig. 12C, the EPSP amplitude is plotted against its 10–90% rise time as measured at the soma for the EPSP generated in each compartmental segment of cell C1–2101, with an identical conductance change. The scatter plot illustrates the strong attenuation of the EPSPs as their rise times increase. The large scatter in the data points is explained by different termination and branching conditions in different dendrites. The filled symbols represent EPSPs generated along one particular dendritic segment (thick den-
drite marked with the arrows in Fig. 12A, *inset*). Figure 12C further illustrates that a wide range of EPSP amplitudes (150 μV to 3.2 mV) can be observed at the soma for synapses of equal strength (and generating a peak amplitude of 3.2 mV when applied at the soma) distributed over the entire dendritic tree. Figure 12D plots the EPSP 10–90% rise time versus half-width as measured in the soma for EPSPs of equal strength generated in every segment of the model. The filled symbols represent the shape index of EPSPs generated on one particular dendrite, highlighted in the inset of Fig. 12A.

**Passive motoneuron**

A long-standing issue in modeling passive motoneurons (as well as other neuron types) has been how to interpret the somatic shunt, and in particular whether this was an artifact of electrode penetration or a genuine low somatic membrane resistivity. Spruston and Johnston (1992) reported that the input resistances of the principal hippocampal neurons were 3–10 times higher when measured by perforated patch compared with intracellular electrodes, indicating the shunt was a result of electrode damage. In other recent whole cell recordings of voltage transients and cable analysis in hippocampal neurons, a somatic shunt has not been required (Major et al. 1994; Thurbon et al. 1994). Similarly, the best fitting results in this investigation did not require a somatic shunt, suggesting a uniform $R_m$ for motoneurons. However, the possibility that $R_m$ (and the other cable parameters) varies along dendrites remains open, as somatic measurements give limited resolution of dendritic membrane properties. More insight into this matter will come from combining somatic and dendritic measurements in the same neuron (Stuart and Spruston 1998).

The values of $R_m$ and $R_i$ confirm previous descriptions of the electrical compactness of motoneuron dendrites for DC signals (Clements and Redman 1989; Fleshman et al. 1988; Ulrich et al. 1994), with dendritic lengths of 0.85 ± 0.14 λ. However, as dendritic attenuation of fast synaptic events is more sensitive to $C_m$ than it is to $R_m$ (Spruston et al. 1994), revising $C_m$ from 1 to 2.4 μF/cm² implies a much greater dendritic attenuation. The example in Fig. 12 indicates peak attenuations of 20–30 for a fast synaptic conductance on the distal dendrites.

A large $C_m$ value has several implications for the integrative behavior of the neuron. It will be more difficult to support an antidromic action potential by inward currents. If a somatic action potential spreads into the dendrite passively, it will attenuate more rapidly. Synaptic potentials generated in fine dendrites will not be as large at their site of generation and will decay more rapidly. This will provide for greater linearity in the summation of synaptic potentials in dendrites. Finally dendritic synaptic potentials will be filtered more heavily, making it more difficult to bring the motoneurons to firing threshold.

We thank M. Hines for indispensable help with the simulator NEURON and Dr. P. Clamann for helpful comments on this manuscript. This work was supported by Swiss National Science Foundation Grant 3100-042055.94 and Swiss Priority Program Grant 5002-037939, both to H.-R Lüscher. Address reprint requests to D. Thurbon.

Received 27 October 1997; accepted in final form 21 January 1998.

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