Estimation of the Electrical Parameters of Spinal Motoneurons Using Impedance Measurements

Mitchell G. Maltenfort and Thomas M. Hamm
Division of Neurobiology, Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona 85013

Submitted 8 September 2003; accepted in final form 13 April 2004

Maltenfort, Mitchell G. and Thomas M. Hamm. Estimation of the electrical parameters of spinal motoneurons using impedance measurements. J Neurophysiol 92: 1433–1444, 2004. First published April 21, 2004; 10.1152/jn.00875.2003. Electrical parameters of spinal motoneurons were estimated by optimizing the parameters of motoneuron models to match experimentally determined impedance functions with those of the models. The model was described by somatic and dendritic membrane resistivities, and the diameter of an equivalent dendritic cable having a standard profile. The impedance functions of motoneurons and optimized models usually differed (rms error) by <2% of input resistance. Consistent estimates for most parameters were obtained from repeated impedance determinations in individual motoneurons; estimates of dendritic resistivity were most variable. The few cells that could not be fit well had reduced impedance phase lag consistent with dendritic penetrations. Most fits were improved by inclusion of a voltage-dependent conductance \( G_V \) with time constant \( \tau_V \). A uniformly distributed \( G_V \) with \( \tau_V > 5 \text{ ms} \) provided a better fit for most cells. The magnitude of this conductance decreased with depolarization. Impedance functions of other cells were adequately fit by a passive model or by a model with a somatic \( G_V \) and \( \tau_V < 5 \text{ ms} \). Most of these neurons (7/8) had resting potentials positive to \(-60 \text{ mV} \). The electrotonic parameters \( \rho, \tau \), and \( L_e \), estimated from model parameters, were consistent with published distributions. Most motoneuron parameters obtained in somatic shunt and sigmoidal models were well correlated, and parameters were moderately affected by changes in dendritic profile. These results demonstrate the utility and limitations of impedance measurements for estimating motoneuron parameters and suggest that voltage-dependent conductances are a substantial component of resting electrical properties.

INTRODUCTION

The importance of a neuron’s electrical characteristics in synaptic integration has motivated considerable effort directed at methods for their determination (reviewed in Jack et al. 1983; Rall 1977; Rall et al. 1992). Typically, system time constant \( (\tau) \), electrotonic length of the dendritic tree \( (L) \), and the dendritic-to-somatic conductance ratio \( (\rho) \) are determined from the voltage transients produced by injection of pulses or steps of current. However, these estimates may be skewed by deviations from idealized neuron properties, like nonuniform membrane resistivity, tapering dendritic trees, and dendritic branches of unequal length (Holmes and Rall 1992a; Holmes et al. 1992; Rose and Dagum 1988). Approaches exist for estimating parameters in neurons with somatic shunts (Durand 1984; Kawato 1984), but obtaining useful estimates often requires the availability of complete morphological information (Clements and Redman 1989; Fleshman et al. 1988; Major et al. 1994; Thurbon et al. 1998) and appropriate constraints (Holmes and Rall 1992b). This requirement limits analysis to relatively few neurons given the long time needed for complete reconstructions.

If reasonable assumptions can be made regarding selected morphological and electrical characteristics of a neuron, the remaining parameters of a suitable neuron model can be determined from recorded responses, providing estimates of electrical properties. This approach is well suited for the use of frequency domain methods. Rall (1960) and Nelson and Lux (1970) explored frequency domain methods theoretically and experimentally (see also Lux 1967), but other attempts to characterize spinal motoneurons in this manner have not been made. The parameters of other neurons have been identified after determination of their impedance and admittance functions (e.g., Moore and Christensen 1985; Moore et al. 1988; Saint Meux and Moore 2000a,b; Tabak et al. 2000; Weckström et al. 1992; Wright et al. 1996).

Changes in the impedance function of a motoneuron can be used to determine the magnitude and location of a sustained conductance change (Maltenfort et al. 2004a,b). The accuracy of these determinations can be improved if estimates of the neuron’s electrotonic parameters are available. This paper describes experimentally determined impedance functions of motoneurons and their use to estimate the electrical parameters of motoneurons.

METHODS

Measurement of impedance

Intracellular recordings were made from motoneurons in pentobarbital anesthetized cats, as described in Maltenfort et al. (2004b). Membrane potentials were recorded during injection of a quasi-white current consisting of a series of evenly spaced sinusoids (2.44–732 Hz). Injected current amplitudes (rms) ranged from 0.2 to 1.7 nA (mean of 1.2 ± 0.5), producing voltage responses (rms) of 0.5 to 1.5 mV (mean of 0.90 ± 0.3). Motoneurons in the current analysis include the cells described in the previous paper plus cells not analyzed in that study because characteristics of recurrent inhibitory postsynaptic potentials (RIPSPs), cumulative impedance change (\( \Delta \Sigma \)), and its variance failed to meet acceptance criteria. Three motoneurons from a separate experiment also were included.

We used discontinuous current clamp (DCC) to minimize the contribution of electrode characteristics to the recordings. Before each motoneuron was impaled, the monitor output of the amplifier was inspected during DCC use. Capacitance compensation and sampling

Address for reprint requests and other correspondence: T. M. Hamm, Division of Neurobiology, Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, 350 W. Thomas Rd., Phoenix, AZ 85013 (E-mail: thamm@chw.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
rate were adjusted to maximize the settling rate of the electrode response without overshoot and allow the response to settle during each cycle of current injection. Minor adjustments were made if needed after impalement. Sampling rates ranged from 2.4 to 7.2 kHz, and most (24/32) were >4 kHz (mean of 4.9 kHz). The anti-aliasing filter was adjusted to minimize noise while minimizing the error from residual electrode voltage caused by current injection (cf. Finkel and Redman 1984). Although these measures would reduce the effect of electrode characteristics on impedance estimations, some residual effect was likely.

The impedance function—the transfer function between injected current and measured voltage—was estimated from the autospectrum of the voltage \( G_c(f) \) and the cross-spectrum of the voltage and current \( G_v(f) \) (Bendat and Piersol 1986).

\[
Z(f) = G_v(f)/G_c(f) 
\]

The impedance estimates were not particularly sensitive to the particular choice of tapering window used to suppress distortion of the power spectra attributed to finite record length (cf. Oppenheim and Schafer 1989; Press et al. 1992). We used a Welch window (Press et al. 1992), which has the advantage of a narrow main lobe in the frequency domain, to minimize overlap between successive spectral points.

The phase of the impedance function was corrected for the delay between A/D channels (one-half the sample period) and the delay introduced by the use of DCC. The later delay depends on DCC sample rate and neuron characteristics and was corrected empirically. Our simulations show that the phase of motoneuron models changes linearly with respect to log (frequency) at the larger frequencies used in our study (Maltenfort et al. 2004a; Fig. 3). A set of phase spectra was constructed for each recorded cell by removing the A/D delay and a range of DCC delays at 10-μs intervals (corresponding to 2.5° at 700 Hz). The most linear phase spectrum from this set between 200 and 700 Hz (based on least error in least-squares linear fit) was used in parameter fits.

The model

Step (somatic shunt) and sigmoidal models were based on 6 morphologically and physiologically identified motoneurons (Cullheim et al. 1987; Fleshman et al. 1988), as described by Maltenfort et al. (2004a). The step model, which we used more often, had a low somatic resistivity \( R_{ms} \), and a larger uniform dendritic resistivity \( R_{md} \), which in the sigmoidal model increased from the value of \( R_{ms} \) proximally to larger values more distally, proportional to cumulative dendritic area. The dendritic tree was represented by a cable with standard dimensions in all motoneurons: diameter \( D_{eq} \) was constant throughout, soma proximally to larger values more distally, proportional to cumulative dendritic area. The dendritic tree was represented by a cable with standard dimensions in all motoneurons: diameter \( D_{eq} \) was constant within 2.5 mm from the soma, beyond which it tapered linearly over 4 mm for a total length of 6.5 mm. Somatic area \( A_s \) was a fourth free parameter.

Model impedance functions were determined using cable equations when the dendrites had uniform resistivity and no voltage-dependent conductances, or using equivalent circuits to represent each compartment when dendritic membrane resistivity was nonuniform and/or contained voltage-dependent conductances (Maltenfort et al. 2004a). Dendritic compartments had electrotonic lengths of 0.05 (cable equation) or 0.01–0.0125 (equivalent circuits); these lengths were chosen to provide a close match between models computed with cable equations and equivalent circuits.

Somatic and dendritic compartments in many models included a voltage-dependent conductance (see RESULTS), represented by a first-order transfer function approximating a Hodgkin–Huxley-type ionic current (Gutman et al. 1974; Moore and Christiansen 1985).

\[
Y_C(\omega) = Y_C(\omega) + G_i/(1 + j \omega \tau_i) 
\]

where \( Y_C(\omega) \) is the admittance (1.0/impedance) of the compartment, \( Y_p(\omega) \) is the admittance of the passive compartment, \( G_i \) is magnitude of the voltage-dependent conductance, and \( \tau_i \) is the time constant describing the kinetics of this conductance. As discussed by Koch (1984), this first-order voltage-dependent term may represent one of several processes, such as activation of an outward current or the inactivation of an inward current with depolarization.

Fitting parametric models to impedance functions

The model parameters \( D_{eq} \), \( A_s \), \( R_{md} \), \( \beta \) \( R_{ms} \), \( Y_P \), \( Y_C \), \( Y_{P}(\omega) \), and \( \nu \) were determined to provide a match between the experimental and model impedance functions. The use of complex impedance functions, including both magnitude and phase, was critical in these determinations. Preliminary simulations and parameter estimations that neglected phase information showed that \( A_s \) and \( R_{ms} \) affect impedance magnitude in a similar manner, so that these two parameters are difficult to fit independently based on magnitude alone.

Parameter estimation used a combined optimization approach to minimize the least-square error between the experimental and model impedance functions. First, simulated annealing from multiple starting points in the parameter space provided an initial randomized search, avoiding local minima that can trap gradient-based optimization. Simulated annealing alone is computationally expensive with convergence criteria not easily defined (Kirkpatrick and Sorkin 1995; Press et al. 1992), so the second phase used a gradient-based optimization to search for a global minimum for the parameter space, a best fit between the model and the experimental impedance function. We used the function “ameba” (Press et al. 1992) for the simulated annealing algorithm, followed by the gradient-based algorithm “frpmn” (Press et al. 1992).

The starting points for simulated annealing were based on systematically perturbed parameter values of model motoneurons based on data of Fleshman et al. (1988); there were 8 different starting points for each of the 6 models. A moderate tolerance (1e-4) was used in these fits. The 12 parameter sets out of the 48 that provided the best fits (least-squared error) were selected, and parameter averages from all sets of 5 in this group of 12 were determined. Least-squared errors between model and experimental impedance functions were recalculated using these parameter averages, and the set of averages that provided the best fit was accepted as the set of final model parameters. This strategy achieved good fits while minimizing computation time with multiple starting points. Parameter variability within the set of 5 used for this average was determined to ensure that parameters were from a neighborhood around the best-fit averages.

Upper and lower bounds were placed on each parameter to ensure that unphysiological parameter values were not selected. To implement these boundary conditions, the parameter set was mapped to a sigmoid function

\[
p_i = b_i + a_i \times [1 - \exp(-2.5 \times y_i)] 
\]

In this equation, \( p_i \) is the value of the ith parameter (e.g., \( R_{ms} \), \( D_{eq} \), etc.); \( b_i \) is the minimum allowable value of the parameter \( p_i \); \( a_i \) is the difference between the maximum and minimum values of \( p_i \); \( y_i \) is the value actually adjusted in the optimization routines. This mapping converts a constrained optimization problem \( (a_i \leq p_i \leq b_i) \) into an unconstrained problem \( (-\infty < y_i < \infty) \) and permits a parameter to approach its bound without encountering a discontinuity, thus ensuring stability (for a related approach, see D’Aguanno et al. 1986).

Bounds for \( R_{md} \) were based on the estimates of Fleshman et al. (1988) and Clements and Redman (1989). Because this data set is small (12 cells), the bounds were extended 2-fold to 3.5–70 kΩ·cm². These studies report \( R_{ms} \) estimates that are much smaller than \( R_{md} \) \( (\approx 10) \), but values of \( \beta \) closer to 1 have been observed (Campbell and Rose 1997). Thus we set the lower bound of \( \beta \) to 1, or \( R_{ms} = R_{md} \), and the upper bound to 999. When using sigmoidal models, ranges of 50–6000 Ω·cm² were used for \( R_{ms} \) and initial \( R_{md} \), and 12.5–108
kΩ-cm² for final $R_{mol}$. These values were extended from those reported by Fleshman et al. (1988).

$D_{eq}$ bounds (16.5 to 62.3 μm) were set to match reported dendritic surface areas (Cullheim et al. 1987; Ulfhake and Cullheim 1988). Somatic surface areas ranged from $2.8 \times 10^{-5}$ cm² (Burke et al. 1982; Ulfhake and Kellerth 1983) because $A_s$ is physiological rather than anatomical and may include part of the proximal dendrites.

Voltage-dependent conductance magnitude $G_v$ was given broad limits: from 0 to 5 times somatic conductance, if somatic, or to 600 μS/cm², if uniformly distributed. The time constant of the voltage-dependent conductance $\tau_v$ was given a range from 0.1 to 75 ms. These limits were determined by the bandwidth of the noise used and the duration of segments of data analyzed. Using a relaxed upper bound for $\tau_v$ (500 ms) in our initial estimations sometimes yielded long values of $\tau_v$ with large $G_v$ values and unrealistically low impedances at frequencies <1–2 Hz. When a pair of conductances was used (see Results), $\tau_v$ limits were set at 0.1–10 and 10–75 ms, respectively.

The optimizations were implemented using a C program on Pentium-based personal computers. Parameter optimization for each impedance record typically took 50 to 60 min for a model with a uniformly distributed voltage-dependent conductance (circuit equations) or 30–40 min for a model with a somatic voltage-dependent conductance (cable equations) run on a computer with a Pentium III processor.

RESULTS

**Parametric fits to impedance functions**

Parametric fits were attempted for 44 impedance functions obtained from 32 motoneurons. Acceptable fits were obtained for 36 impedance functions (25 motoneurons), with error $<40$ kΩ or $<2.5\%$ input resistance (rms error, square-root of average squared difference between model and measured impedance functions). A 4-parameter passive model was less satisfactory for many neurons than models that included a voltage-dependent conductance. The impedance magnitude of passive models declines monotonically with frequency, but the magnitude of experimental impedance functions often (23 of 44) exhibited a short rise before starting to decline at 10–20 Hz (Fig. 1A). The phase of impedance functions with this characteristic also displayed a small lead at low frequencies (Fig. 1B), unlike the passive models. These characteristics were adequately fit using a model that included a voltage-dependent conductance ($G_v$; dotted lines in Fig. 1). The impedance function of this model, the impedance function of this model with the effect of $G_v$ removed (dashed-dotted line), and the impedance function fit to a passive model (dashed line) coincided at frequencies >100 Hz. Impedance estimates were less certain at the low frequencies in which $G_v$ effects were greatest, as indicated by lower coherence values (Fig. 1C), but the common occurrence of these effects indicates they are genuine features of motoneuron impedance. The remaining impedance functions tended to be flatter, with slight leads or smaller lags at low frequencies than predicted by the passive models, and models with $G_v$ better described many of these.

Figure 1 gives an example of one of the best fits (cell 11, type FF, rms error = 4.7 kΩ, 0.7% of $R_{mol}$). The mean rms error

**FIG. 1.** Comparison of experimental impedance functions with best-fit model representations. A and B: magnitude and phase, respectively, of the measured impedance function of a motoneuron (solid line; cell 11, type FF) and the impedance functions for 2 best-fit models. Dotted line shows the impedance function for a model incorporating a uniformly distributed voltage-dependent conductance ($G_v$). Effect of removing this conductance from the model is shown by the dashed-dotted line. Best fit achieved without use of a voltage-dependent conductance is given by the dashed line. Coherence for the experimental impedance function is given in C, showing that voltage and current were highly correlated throughout the spectrum, although, typically, coherence was less at low frequencies.
for acceptable fits was 11.9 kΩ (1.0%). Examples of impedance magnitude and phase for an average fit (cell 25, type FF, rms error = 11.3 kΩ, 1.1%) and for one of the worst acceptable fits (cell 22, type FF, rms error = 19.5 kΩ, 2.1%) are shown in Fig. 2, A and B and C and D, respectively.

Acceptable fits were not obtained for 8 impedance functions from 7 motoneurons, even with models that included one or 2 voltage-dependent conductances. These impedance functions were distinguished by larger impedance magnitudes, smaller phase lags at higher frequencies, $A_s$ estimates near the lower boundary (2.8e-5 cm$^2$), and smaller $R_{ms}$ estimates. Similar impedance functions were observed in simulations of dendritic recordings, using a model with 2 dendritic cables in which input impedance was determined in the first dendritic compartment of the smaller cable.

Impedance functions with acceptable fits included 10 (measured from 8 motoneurons) with minimum $A_s$ values. The mean phase lag at 450 Hz in this group was $-33.9 \pm 4.5^\circ$, compared with $-39.7 \pm 5.1^\circ$ for other motoneurons with acceptable fits and $-23.7 \pm 4.8^\circ$ for cells without good fits. Cells with impedance functions that required minimal $A_s$ values also had small $R_{ms}$ estimates and large $R_{md}$ estimates, possibly as a result of adjustments by the optimization algorithms to compensate for limiting values of $A_s$. These cells were excluded from the following analysis, which was based on 26 impedance functions from 17 motoneurons.

We compared fits obtained with a passive model, a model with a voltage-dependent conductance restricted to the soma, and a model with a voltage-dependent conductance uniformly distributed through the neuron. A 10% reduction in error was set as a criterion for one fit to be better than another. Smaller differences did not appear to provide meaningful discriminations. Somatic voltage-dependent models provided better fits for 20 of 26 cases with an average improvement of 31.3 ± 23.8%. Errors for the somatic and uniform voltage-dependent models were generally similar (Fig. 3). The time constant of the voltage-dependent conductance ($\tau_v$) was longer (9.5–71 ms) for the 8 cases in which the uniform models provided better fits, and shorter (0.9–3.3 ms) models provided better fits for 20 of 26 cases with an average improvement of 31.3 ± 23.8%. Errors for the somatic and uniform voltage-dependent models were generally similar (Fig. 3). The time constant of the voltage-dependent conductance ($\tau_v$) was longer (9.5–71 ms) for the 8 cases in which the uniform models provided better fits, and shorter (0.9–3.3 ms)}
whereas motoneurons with a short time constant confined to the soma and one with longer time constant was uniformly distributed, did not provide better fits than models with a single voltage-dependent conductance, except in one case (reducing rms error by 17%). In the set of 26 impedance functions accepted for full analysis, passive models were most appropriate for 4, models with uniformly distributed voltage-dependent conductances for 18, models with somatic voltage-dependent conductances for 3, and a dual conductance model for one.

**Distribution of model parameters**

The 4 parameters describing the passive electrical structure of the motoneuron, were skewed to lower values (Fig. 4), D_{eq} presenting the most normal distribution (Fig. 4A). A_{s} estimates (Fig. 4B) included values higher than suggested by the published anatomical measurements, ranging from 1.25 to 8.9% of dendritic area (mean of 3.52 ± 1.86%). These relatively large somatic area estimates probably include juxtasomatic regions of proximal dendrites that are effectively isopotential with the soma. R_{ms} values (Fig. 4C) tended to be low, although one large estimate was obtained, in a type S, soleus motoneuron. R_{ms} estimates (Fig. 4D) ranged widely and included values higher than those of Fleshman et al. (1988), but comparable to the estimates of Clements and Redman (1989). The distributions of R_{md} and A_{s} were quite similar for motoneurons of different motor unit type (as determined by input resistance and rheobase). D_{eq} tended to be smaller in FR than in FF units (24.4 ± 5.3 vs. 27.9 ± 6.7 μm), and R_{ms} tended to be smaller in FF than in FR units (181 ± 102 vs. 258 ± 138 Ω·cm⁻²), but these tendencies were not significant (t = 1.39, P = 0.177 and t = 1.50, P = 0.149, respectively).

The only passive parameter correlated with resting potential was R_{ms}, which tended to increase with depolarization (Fig. 5A; r = 0.42, t = 2.22, P = 0.04). This correlation suggests the presence of a somatic voltage-dependent conductance with very short time constant, indistinguishable from passive conductance with the bandwidth of injected current used in these experiments. An inward current activated with depolarization, like subthreshold sodium current or persistent sodium current, would produce the observed correlation between R_{ms} and resting potential. Although an increase in R_{ms} with activation of a conductance seems paradoxical, an inward current would decrease the slope conductance at depolarized membrane potentials, yielding larger R_{ms} estimates.

G_{V} estimates for uniformly distributed conductances ranged from 36 to 247 μS/cm² (mean of 102.4 ± 59.1; Fig. 5B). These values were large in relation to dendritic conductance, averaging 196 ± 151% of 1/R_{md}. Somatic G_{V} values associated with short time constants were also substantial, ranging from 57 to 221 nS (mean of 131 nS; not shown). The uniformly distributed G_{V} decreased in size with depolarization (r = −0.51, t = −2.40, P = 0.03). Moreover, 3 of the 4 cells described by passive models and each of the cells with τ_{V} values <5 ms had resting potentials more positive than −60 mV (Fig. 5C). τ_{V} values (in all cells with G_{V} terms) were not significantly correlated with resting potential (Fig. 5C; r = −0.31, t = −1.44, P = 0.17). Overall, this analysis indicates that the experimentally determined impedance functions include the contributions of one or more uniformly distributed conductances at hyperpolarized resting potentials that inactivate with depolarization.

The consistency of parameter estimates can be judged by repeated estimates obtained from fits to different impedance functions from the same motoneuron, linked by lines in Fig. 5, A–C. (The separate records used to compute different impedance functions were obtained during tests of different sources of recurrent inhibition; Maltenfort et al. 2004a.) The same model (i.e., the same G_{V} distribution) provided the best fit in 6 of the 3 cases in which the fit was better using the somatic model. We proceeded with the assumption that motoneurons with τ_{V} <5 ms were described better by the somatic model, whereas motoneurons with τ_{V} greater than this value were described better by the uniform model.

Models with 2 voltage-dependent conductances, one with a short time constant confined to the soma and one with longer time constant that was uniformly distributed, did not provide better fits than models with a single voltage-dependent conductance, except in one case (reducing rms error by 17%). In the set of 26 impedance functions accepted for full analysis, passive models were most appropriate for 4, models with uniformly distributed voltage-dependent conductances for 18, models with somatic voltage-dependent conductances for 3, and a dual conductance model for one.

**FIG. 4.** Distribution of model parameters. These histograms show distributions of the diameter of the equivalent dendritic cylinder (D_{eq}; A), somatic area (A_{s}; B), specific resistivity of the somatic membrane (R_{ms}; C), and specific resistivity of the dendritic membrane (R_{md}; D). The motor unit type of each cell (classified by input resistance and rheobase) is indicated.
of 9 of these comparisons. In the other 3 comparisons the best model changed from a passive- to a somatic-conductance model, or from a uniform-conductance to a passive-conductance model. Each of these 3 cases was associated with depolarization to a resting potential more positive than −60 mV.

Most changes in $A_\nu$, $D_{eq}$, and $R_{ms}$ between same-cell estimates were small compared with the range of population values and are consistent with the small changes (5–10% of magnitude) observed between 2 impedance functions from single motoneurons (Fig. 6, A and B). $R_{md}$ estimates show greater variability (Fig. 6B). Figure 6 demonstrates a correlation between $A_\nu$ and $D_{eq}$ ($r = 0.61$, $t = 3.72$, $P = 0.001$) and suggests a negative correlation between $R_{ms}$ and $R_{md}$ ($r = -0.38$, $t = 2.03$, $P = 0.05$ for all cells; $r = -0.52$, $t = -2.90$, $P = 0.008$ without the outlier).

Distribution of electrotonic parameters ($\tau$, $\rho$, and $L$)

Electrotonic parameters (Fig. 7) were determined from the passive model parameters. Dendritic-to-somatic conductance ratio, $\rho$, was the ratio between the steady-state ($f = 0$ Hz) impedance of the somatic compartment and that of the dendritic cylinder [i.e., $\rho = Z_s(0)/Z_d(0)$]. The system time constant $\tau$ was based on the membrane-weighted average of somatic and dendritic membrane time constants, with an empirical correction for $\rho$ (Fleshman et al. 1988). $L$ was defined by the location of the dendritic compartment where the cumulative dendritic membrane area reached 97% of the total dendritic membrane area, corresponding to the conductance-weighted electrotonic length $L_G$, of Fleshman et al. (1988). Corresponding values from the studies of Fleshman et al. (1988) and Clements and Redman (1989) are plotted in Fig. 7 for comparison. The ranges of $\rho$, $\tau$, and $L$ were similar to those found in previous studies, although we found a broader range of values. Several estimates of $L$ exceeding 2 were associated with lower values of $R_{md}$. All but one $\rho$ value was $<1$; this value (2.9) occurred in the type S motoneuron with large $R_{ms}$. Differences between repeated estimates of $\rho$, $\tau$, and $L$ for the same motoneuron (not shown) varied by an extent similar to that found for repeated $R_{md}$ estimates (Fig. 6B).

No significant differences were found between different neuron types in $\tau$ (FF: 7.8 ± 0.9 ms; FR: 8.5 ± 1.0 ms; S: 6.1 ± 0.3 ms), $\rho$ (FF: 0.29 ± 0.06; FR: 0.34 ± 0.06 ms; S: 1.67 ± 1.26), or $L$ (FF: 1.65 ± 0.13; FR: 1.85 ± 0.16 ms; S: 2.25 ± 0.15). Comparisons were limited by the small sample of 2 type S motoneurons.

Dependency of parameters on assumed motoneuron electrical and morphological structure

Parameters also were determined for sigmoidal models and for models with altered cable structures to assess the effect of model assumptions on parameter estimates. Sigmoidal models, in which membrane resistivity increases monotonically from soma through dendrites, can produce electrical behavior identical to that of step models (Fleshman et al. 1988; Segev et al. 1990). Two sigmoidal models were used, one with a uniformly...
distributed voltage-dependent conductance, the other with a voltage-dependent conductance that was proportional to $1/R_{md}$. The model with a uniform $G_V$ provided better fits, on average, than the proportional model, although this difference was not significant (paired $t$-test; $t = 2.02, P = 0.05$). The proportional model produced a better fit (rms error reduced by $10\%$) in only one cell; the best step-model description of this cell had a somatic voltage-dependent conductance.

Step models provided slightly better fits than sigmoidal models with uniform voltage-dependent conductances, on average, with $91\%$ of the rms error of the latter (paired $t$-test, $t = 2.43, P = 0.03$). Estimates of each of the 4 passive parameters obtained with the 2 models were strongly correlated: $A_s (r = 0.98)$, $D_{eq} (Fig. 8A; r = 0.99)$, $R_{md} (Fig. 8B; r = 0.82)$, and $R_{ms} (r = 0.90)$. As found by Fleshman et al. (1988), $R_{ms}$ in the sigmoidal model was approximately 2-fold larger. Sigmoidal estimates of $A_s$ and $D_{eq}$ were approximately 90 and $110\%$, respectively, of the corresponding step-model estimates. Estimates of $L$ (Fig. 8C) and $\rho$ were well correlated ($r = 0.89, r = 0.86$, respectively); correlations between $\tau$ estimates were

**FIG. 6.** Parameter variability and correlations between passive model parameters. In the scatterplots of A and B, estimates based on successive sets of impedance records from the same motoneuron are linked by lines, as in Fig. 5. A plots $D_{eq}$ vs. $A_s (r = 0.61)$. B shows $R_{ms}$ and $R_{md} (r = -0.38)$. Inverse correlation between $R_{ms}$ and $R_{md}$ was strengthened by removal of the outlying value of $R_{ms} (r = -0.52)$.

**FIG. 7.** Distribution of estimates of time constant ($\tau$), dendritic-to-somatic conductance ratio ($\rho$), and electrotonic length ($L$). Black bars refer to putatively identified type FF motoneurons, the gray bars to FR motoneurons, and the open bars describe type S motoneurons. For comparison, values of $\tau$, $\rho$, and $L$ from Fleshman et al. (1988) and values of $\tau$ and $\rho$ from Clements and Redman (1989) are also plotted in these histograms, as indicated. Values of $L$ given are based on "$L_g$" in Fleshman et al. (1988). Estimates provided by Fleshman et al. (1988) are identified by type FF, FR, or S.
weaker ($r = 0.65$). Both $G_V$ and $\tau_V$ estimates in the 2 models were highly correlated ($r = 0.97$ and $r = 0.95$, respectively).

Clements and Redman (1989) reported that parameter estimates were sensitive to changes in the length of the equivalent cylinder, particularly estimates of $R_{md}$. We examined the effect on parameter estimates of models with altered dendritic profiles in a subset of 6 motoneurons selected to provide a range of parameter values. Dendritic profiles were altered by shortening the uniform or the tapered portion of the equivalent cable by 15% (1 mm), decreasing membrane area by 31 and 19%, respectively. For 5 of 6 motoneurons, $R_{md}$ estimates were less in shortened dendrites (average decrease of 22%; Fig. 8D), although results were variable (range +12 to −67%). These results are generally consistent with those of Clement and Redman (1989), who observed changes as large as 20–30% in dendritic resistivity when the constant-diameter dendritic region was reduced by 0.1 mm. This variability in $R_{md}$ resembles that found for repeated estimates in individual motoneurons (cf. Fig. 6). In contrast, most estimates of equivalent diameter (Fig. 8E), somatic area, and $R_{ms}$ obtained in shortened-cable models were within 10% of standard values. Changes in $G_V$ were moderate and variable, but $\tau_V$ estimates increased by an average of 83% (range of 26 to 230%) when the constant-diameter was shortened. Changing the taper produced smaller effects (−4 to 44%).

Estimated time constant was decreased by 23% (range 2 to −51%) when the constant-diameter region was shortened and by 19% (range −11 to −29%) when the tapering region was changed, similar to changes in $R_{md}$. $\rho$ consistently increased with shortened tapers but was less predictable when the constant-diameter segment was shortened (Fig. 8F). Changes in $L$ were variable but usually <20% of the standard value.

**Discussion**

The results of this study demonstrate the feasibility of estimating neuron parameters using impedance functions determined from in vivo recordings. Frequency-domain techniques for parameter estimation provide some advantages over more frequently used time-domain methods, including greater immunity to the effects of noise and nonlinearities (Fu et al. 1989; Wright et al. 1996). Several findings support the reli-
ability of parameter estimates in this study, including their similarity to estimates of previous studies, their relative insensitivity to choice of model, and the dependency of voltage-dependent terms on resting potential. However, the use of this approach is subject to several limitations.

Methodological issues

Our estimated parameters are subject to some uncertainty attributable to incomplete compensation for electrode properties. Accurate compensation for electrode capacitance and adjustment of sampling parameters pose significant difficulties (Wilson and Park 1989). One approach to this problem is to include electrode parameters in the optimization procedures (e.g., Saint Mleux and Moore 2000a; Wright et al. 1996). With the potential for electrode polarization and complex characteristics when recording from relatively deep tissue, we elected to use discontinuous current clamp (DCC) despite its attendant uncertainties (cf. Campbell and Rose 1997). With the sampling rates in this study (mean of 4.9 kHz), part of the motoneuron response may have decayed during each cycle of current injection, reducing impedance estimates throughout the frequency range. The parameters most directly affected by this error would be \( R_{\text{ms}} \) and \( A_{c} \), given that size parameters and membrane capacitance are the primary determinants of impedance at higher frequencies. Incomplete settling of the electrode response would, on the other hand, increase estimated impedance. Use of higher sampling rates and electrodes with faster response would, on the other hand, increase estimated impedance.

The use of DCC added an additional phase delay that appeared to depend on sampling rate and cell characteristics. Correction for this factor is necessary because phase information is needed to distinguish the effects of \( R_{\text{ms}} \) and \( A_{c} \) on impedance and estimate these parameters (see METHODS). We used an empirical approach, subtracting a delay sufficient to produce a phase profile at high frequencies that resembled model impedance phase functions. This procedure undoubtedly left some error, but the similarity in phase profiles of recorded and model neurons (cf. Figs. 1 and 2 with Fig. 3 of Maltenfort et al. 2004b) suggests that this error is relatively small. Any errors would affect \( R_{\text{ms}} \) directly, given that \( R_{\text{ms}} \) determines the frequency at which phase is \( -45^\circ \) (Maltenfort et al. 2004b), and \( A_{c} \) indirectly, to compensate for the \( R_{\text{ms}} \) error.

\( R_{\text{ms}} \) estimates displayed the greatest variability in repeated estimates of individual cells. White et al. (1992) observed that the determination of dendritic parameters in a neuron with a somatic shunt is ill posed, sensitive to small errors. The variability of repeated \( R_{\text{ms}} \) estimates of model motoneurons with physiological amounts of noise is substantial and consistent with this observation (T. M. Hamm, unpublished observations). Although accuracy can be improved by anatomical constraints, \( R_{\text{ms}} \) estimates are inherently the least reliable. Consequently, estimates of \( \tau \), \( p \), and \( L \), dependent on \( R_{\text{ms}} \), are subject to error.

Model assumptions

Spinal motoneurons possess several features that violate assumptions used in Rall’s original procedures for estimating electrotonic parameters in neurons (Rall 1969, 1977), including somatic shunts (Barrett and Crill 1974; Clements and Redman 1989; Fleshman et al. 1988; Iansek and Redman 1973a; Rose and Vanner 1988; cf. however, Nitzan et al. 1990), and by tapering dendrites and dendrites of unequal length (Barrett and Crill 1974; Bras et al. 1987; Cameron et al. 1985; Fleshman et al. 1988; Kernell and Zwaagstra 1989; Redman and Walmsley 1983; Rose et al. 1985; Ulfhake and Kellerth 1981). Consequently, we based our estimates on a model that incorporated these features, as other investigators have in estimation and modeling studies (e.g., Durand 1984; Holmes and Rall 1992a; Kawato 1984; Powers and Binder 1996). However, substantial errors can be introduced by variation of these characteristics (Holmes et al. 1992; Rose and Dagum 1988; White et al. 1992). In general, the electrotonic parameters of a neuron cannot be uniquely determined without a complete morphological description (Holmes and Rall 1992b), and several investigators have noted that similar fits to experimental data can be provided with different parameters (e.g., Ali-Hassan et al. 1992; Rose and Dagum 1988). Our parameter estimates may be influenced by departures from model assumptions.

Our models used a standard cable structure based on tapering dendritic profiles found by Fleshman et al. (1988) and Clements and Redman (1989). These profiles were rather similar within the small number of motoneurons in each study, but differed between the 2 studies. Although dendritic geometries of motoneurons in different muscle systems vary (Rose et al. 1985), lumbosacral motoneurons exhibit rather similar geometries with few exceptions (Ulfhake and Kellerth 1983). This uniformity of dendritic geometry in different lumbosacral motoneuron pools is consistent with the assumption of a standard dendritic profile, but electrotonic profiles of the dendrites of different species of motoneurons have not been compared. Moreover, the dendritic trees of type F motor units are more expansive than those of type S units (Cullheim et al. 1987; Gustafsson and Pinter 1984; Ulfhake and Kellerth 1982), raising the possibility that motoneurone of different types may be better represented by models with different dendritic profiles.

We observed a moderate sensitivity of parameter estimates to changes in cable profile (Fig. 8). The greater sensitivity observed by Clements and Redman (1989) may result from differences in the 2 studies: the shorter dendritic profile used by Clements and Redman, the use of time-domain rather than frequency-domain methods, or the use of a fixed soma as opposed to estimating soma area with the other parameters. Regardless of the reason for the different sensitivities, both studies indicate that parameter estimation should be accompanied by an analysis of parametric sensitivity to model assumptions.

Parameters obtained with step and sigmoidal models were strongly correlated. The choice of model affected \( A_{c} \) and \( D_{\text{eq}} \) estimates by about 10%, in addition to the expected influence on \( R_{\text{ms}} \). Step models provided slightly better fits on average than sigmoidal models, suggesting that the combination of somatic shunt with uniform voltage-dependent conductance is a better representation for most motoneurons than a sigmoidal distribution of resistivity and uniform \( G_{V} \). The similarity of fit provided by different models implies that alternative models, \( G_{V} \) distributions weighted toward the soma or dendrites, for
example, are unlikely to improve the goodness of fit or substantially affect the parameters.

Parameter estimates are also dependent on $R_i$ and $C_m$. Previous studies support the values of $70 \ \Omega \cdot \text{cm}$ (Barrett and Crill 1974; Clements and Redman 1989; Stuart and Spruston 1998; Thurbon et al. 1998) and $1 \ \mu\text{F/cm}^2$ (Major et al. 1994; Ulrich et al. 1994; Wright et al. 1996) used for $R_i$ and $C_m$, respectively. However, several studies have provided larger estimates of $R_i$ in different kinds of neurons (e.g., Major et al. 1994) as well as a range of $C_m$ values (Barrett and Crill 1974; Nitzan et al. 1990; Thurbon et al. 1998). Our parameter estimates are subject to these uncertainties.

**Distribution of motoneuron parameters**

Motoneurons of different type and size differ in some parameters, including membrane resistivity and system time constant (Burke et al. 1982; Fleshman et al. 1988; Gustafsson and Pinter 1984; Kernell and Zwaagstra 1981; Zengel et al. 1985). We found that $R_{\text{ms}}$ tended to be greater in FR than in FF motoneurons, consistent with previous work. $R_{\text{ms}}$ did not differ between cell types; the uncertainty in this parameter may have obscured any differences, if present.

The low $R_{\text{ms}}$ values found in this study are characteristic of neurons with a somatic shunt. Uniform membrane resistivities are sufficient to characterize whole cell recordings from ventral horn neurons in slice (Thurbon et al. 1998), suggesting that the somatic shunt arises fully from damage. However, $R_{\text{ms}}/R_{\text{ns}}$ is decreased in cesium-loaded motoneurons (Campbell and Rose 1997), indicating that voltage-dependent potassium channels contribute to the somatic shunt. Although inclusion of voltage-dependent parameters in the identification process should reduce the effect of voltage-dependent conductances on estimates of membrane resistivity, our estimates may have been affected by conductances active at rest. The dependency of $R_{\text{ns}}$ on resting potential found in this study (Fig. 5A) suggests that voltage-dependent conductances contribute to somatic resistivity.

A voltage-dependent conductance was required for most cells, and $G_V$ was often substantial. The uniformly distributed $G_V$ found in most cells decreased with depolarization, and $\tau_V$ averaged 39 ms, although the range of values was broad. The hyperpolarization-activated mixed cation current, $I_h$, which is present in motoneurons (Barrett et al. 1980; Bayliss et al. 1994; Chandler et al. 1994; Takahashi 1990a,b), has properties consistent with $G_V$ and likely contributes to this conductance. $I_h$ channels are distributed in the dendrites as well as the soma of neonatal rat motoneurons (peripheral with a dendritic dominance; Kjaerulf and Kiehn 2001). Motoneurons with shorter afterhyperpolarizations (AHP; larger cells) exhibit greater amounts of sag (Gustafsson and Pinter 1984), attributable to $I_h$. Considering AHP durations in motoneurons of different type (Zengel et al. 1985), differences in sag and $I_h$ should be greater between types F and S than between types FF and FR motoneurons. Uniformly distributed $G_V$ averaged $130 \pm 68$ and $82 \pm 43 \ \mu\text{S/cm}^2$ ($t = 1.72, P = 0.11$) in the FF and FR cells of our sample, respectively.

Several neurons with resting potentials $> -60 \ \text{mV}$ were best described by models having a fast voltage-dependent conductance localized to the soma. Potassium conductances like $I_h$ and $I_{\text{ksfr}}$ are present in motoneuron somata (Safronov and Vogel 1995; cf. Campbell and Rose 1997) and could contribute to this somatic conductance, although these conductances appear to be present in dendrites as well (Clements et al. 1986). Characteristics of these conductances are consistent with results of this study, but firm conclusions cannot be made. Impedance measurements at a single mean membrane potential have limited ability to characterize voltage-dependent currents; more information can be obtained from measurements at multiple potentials (Saint Mleux and Moore 2000a,b; Tabak et al. 2000).

Other conductances may have contributed to the characteristics of the impedance functions, including $\text{Ca}^{2+}$-activated $K$ conductances (Barrett et al. 1980; Takahashi 1990a,b; Umemiya and Berger 1994) and persistent inward $Na^+$ conductance (Chandler et al. 1994; Lee and Heckman 2001; Powers and Binder 2003). The size of the contribution made by voltage-dependent terms in our estimates implies that voltage-dependent conductances make substantial contributions to subthreshold behavior in lumbar sacral motoneurons. The abundance of mechanisms for modulating these conductances in motoneurons (Powers and Binder 2001; Rekling et al. 2000) implies considerable potential for control of synaptic integration in motoneurons.

**Acknowledgments**

We thank T. Fleming for technical assistance and Drs. R.E.W. Fyffe and P. K. Rose for comments on an early draft of this work. We also thank the journal’s anonymous referees for constructive, helpful comments.

Present address of M. G. Maltenfort, Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129.

**Grants**

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-22454 to T. M. Hamm and NS-07309 to the University of Arizona—Barrow Neurological Institute Motor Control Neurobiology Training Program. M. G. Maltenfort received support from NS-10341.

**References**


