Determination of the Location and Magnitude of Synaptic Conductance Changes in Spinal Motoneurons by Impedance Measurements

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Maltenfort, Mitchell G., Carrie A. Phillips, Martha L. McCurdy, and Thomas M. Hamm. Determination of the location and magnitude of synaptic conductance changes in spinal motoneurons by impedance measurements. J Neurophysiol 92: 1400–1416, 2004. First published April 21, 2004; 10.1152/jn.00873.2003. The relation between impedance change and the location and magnitude of a tonic synaptic conductance was examined in compartmental motoneuron models based on previously published data. The dependency of motoneuron impedance on system time constant (τ), electrotonic length (L), and dendritic-to-somatic conductance ratio (ρ) was examined, showing that the relation between impedance phase and ρ differed markedly between models with uniform and nonuniform membrane resistivity. Dendritic synaptic conductances decreased impedance magnitude at low frequencies; at higher frequencies, impedance magnitude increased. The frequency at which the change in impedance magnitude reversed from a decrease to an increase—the reversal frequency, F_r—was a good estimator of electrotonic synaptic location. A measure of the average normalized impedance change at frequencies less than F_r, cuΔZ, estimated relative synaptic conductance. F_r and cuΔZ provided useful estimates of synaptic location and conductance in models with nonuniform (step, sigmoidal) and uniform membrane resistivity. F_r also provided good estimates of spatial synaptic location on the equivalent cable in both step and sigmoidal models. Variability in relations between F_r, cuΔZ, and conductance location and magnitude between neurons was reduced by normalization with ρ and τ. The effects on F_r and cuΔZ of noise in experimental recordings, different synaptic distributions, and voltage-dependent conductances were also assessed. This study indicates that location and conductance of tonic dendritic conductances can be estimated from F_r, cuΔZ, and basic electrotonic motoneuron parameters with the exercise of suitable precautions.

INTRODUCTION

The position of a synapse in the dendritic arbor of a neuron is a critical factor in its contribution to synaptic integration (Rall 1964). Within the dendrites of spinal motoneurons, synaptic locations have been identified through arduous reconstructions of single motoneurons and presynaptic neurons after intracellular staining (e.g., Brown and Fyffe 1981; Burke and Glenn 1996; Burke et al. 1979; Fyffe 1991; Redman and Walmsley 1983). The electrotonic positions of synapses have been also identified using the shapes of the postsynaptic potentials produced by single afferents (Iansek and Redman 1973; Jack et al. 1971; Mendell and Henneman 1971; Rall et al. 1967; Redman and Walmsley 1983). These determinations depend on the satisfaction of several conditions, such as having reasonable estimates of the duration of synaptic current and knowledge of the electrotonic characteristics of the motoneuron (Jack et al. 1975), and, of course, the ability to obtain suitable recordings from pairs of neurons for the electrophysiological measurements.

Considering the inherent difficulties and limitations of these methods, it is not surprising that our knowledge of the synaptic organization in the dendrites of motoneurons is still limited. An alternative method for determining synaptic location through electrophysiological measurements was proposed by Fox (1985). Fox demonstrated through compartmental models that the impedance function Z(f) of a neuron, the ratio of the amplitude of voltage response to the amplitude of injected sinusoidal current of frequency f, varies in a systematic way with the location of a synapse during its tonic activation. Fox and Chan (1985) confirmed the utility of this method in recordings from cultured neurons, but no further application has been made in experimental investigations.

The goal of this study was to develop the impedance measurement for application in studies of spinal motoneurons. We sought to use impedance functions to determine both the location and the magnitude of the conductance change produced by a steady synaptic input. The magnitude of this conductance change is critical for assessing the contribution of the synapse to dendritic integration, but is not readily obtained in most morphological studies.

We examined how the electrotonic parameters of a motoneuron, different models of nonuniform membrane resistivity, and voltage-dependent conductances affect both a motoneuron’s impedance function and the changes in impedance created by synaptic inputs. In a companion paper (Maltenfort et al. 2004), we describe the application of this method to the synaptic conductances produced by recurrent inhibition. Preliminary accounts of this work have been published (Hamm and McCurdy 1992; Hamm et al. 1993).

METHODS

We explored the dependency of the impedance functions of motoneurons on their electrotonic parameters and synaptic location and conductance, using compartmental models. Most simulations were based on parameters provided in the study of Fleshman et al. (1988), which were based on electrophysiological measurements and anatomical reconstructions of 6 type-identified triceps surae α-motoneurons (Cullheim et al. 1987). The following will describe how each model was constructed and used to determine the sensitivity of impedances and synaptic effects to changes in the electrotonic parameters of the neuron.

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Motoneuron models

Fleshman et al. (1988) found that 2 models with nonuniform membrane resistivity provided equally good fits to their results, obtained using sharp electrodes. In the somatic shunt (or “step”) model, the dendritic resistivity was uniform, but larger than somatic resistivity. In the “sigmoidal” model, the dendritic resistivity increased monotonically with distance from the soma, proportionately with the percentage of dendritic membrane area. Previous investigations have used either the somatic shunt model (e.g., Clements and Redman 1989; Fu et al. 1989; Jones and Bawa 1997; Rapp et al. 1994; Segev et al. 1990) or the sigmoidal model (Powers and Binder 1996) to represent the electrotonic properties of motoneurons.

This paper and its companions (Maltenfort and Hamm 2004; Maltenfort et al. 2004) focus on the somatic shunt model, which facilitates comparison to other studies of motoneuron properties and synaptic location (cf. Burke and Glenn 1996; Clements and Redman 1989; Fyffe 1991). Some simulations were performed using the sigmoidal model or a model with uniform membrane resistivity (i.e., without a somatic shunt) for purposes of comparison. Motoneuron models were uniquely defined by \( R_{\text{ms}} \) and \( R_{\text{md}} \), the somatic and dendritic specific resistivities; \( A_c \), somatic area, determined from \( r \), the dendritic-to-somatic conductance ratio, and the conductance of the dendritic cable; \( D_{\text{eq}} \), the initial diameter of the equivalent cable, at the first dendritic compartment; \( R_c \), cytoplasmic resistivity; and \( C_{m'} \), specific membrane capacity. Values of 70 \( \Omega \cdot \text{cm} \) and 1 \( \mu \text{F/cm}^2 \) were used for \( R_c \) and \( C_{m'} \), respectively. The other parameters for each neuron were determined according to values provided by Fleshman et al. (1988; Table 5 and Fig. 9 therein).

The motoneuron dendritic tree was modeled as a single tapered equivalent cable, consisting of a series of isopotential compartments (Fig. 1A; see Rall 1977 for underlying theory). The electrotonic length of each compartment \( L_c \) was 0.05 in the step model and 0.01 in the sigmoidal model. The shorter compartment length in the sigmoidal model was used to reduce the variation in membrane resistivity within compartments. We used a “standard profile” for the equivalent cable of each motoneuron based on Fig. 9 in Fleshman et al. (1988; see also Clements and Redman 1989); the cable diameter \( D_c \) was constant for 0.25 cm from the soma, and then decreased linearly over the next 0.4 cm. The diameter of the proximal 0.25 cm segment in each model \( D_{\text{eq}} \) was taken from the Fig. 9 legend of Fleshman et al. (1988). Starting at the soma, the cable length of each dendritic compartment \( l_c \) (in cm) was determined using the equation \( l_c = L_c \times \lambda = L_c \times (R_{\text{ms}}D_c / 4R_c)^{\rho} \), where \( \lambda \) is the cable length constant. Once the cumulative cable length 1 exceeded 0.25 cm, \( D_c \) was determined by \( D_c = D_{\text{eq}} - D_{\text{eq}}(l - 0.25)/0.4. R_{\text{ms}} \) was a constant in the step model, but varied by compartment in the sigmoidal model and was defined as \( R_{\text{ms}} + C u A(l_1) \times (R_{\text{max}} - R_{\text{ms}}) \), where \( C u A(l_1) \) is the cumulative fraction of total dendritic membrane area between the soma and the current compartment, and \( R_{\text{max}} \) is the maximum value for \( R_{\text{md}} \).

Determination of impedance functions

Once the parameters were determined for all dendritic compartments, impedance functions were determined using equations from Fox (1985). These equations for impedance can be derived from the solution to the cable equation (e.g., Rall 1977). A succinct presentation of this derivation is given by Yang and Chapman (1983).

The “characteristic impedance” at the end of a semi-infinite cable is defined as

\[
Z_c(\omega) = [2/\pi q(\omega)](R_{\text{ms}}R_c)^{\rho} D_c^{-\rho} \quad (1a)
\]

where \( q(\omega) = (1 + \sigma_0 r_0)^{1/2} \); \( \omega \) is angular frequency; and \( \sigma_0 \) is the time constant of the dendritic membrane, the product of \( R_{\text{md}} \) and the membrane capacitance per unit area.

Using characteristic impedance, the input impedance of a finite cable of length \( L_c \) under different boundary conditions can be expressed as (Fox 1985; Rall 1977)
The impedance of the entire equivalent dendrite arises from the nonlinear sum of the individual compartment impedances. The admittances \( Y_{i,m} \) and \( Y_{i,k,clp} \) are the reciprocals of the impedances defined in Eq. 1 above. Define \( Y_{i+1,0} \) as the combined admittance of contiguous compartments 0 to \( i \), 0 being the index of the most distal compartment. Then the admittance of the entire equivalent dendrite, as seen from the soma, arises from iterative application of the following equation

\[
Y_{0...k} = \left[ Y_{i+1,0} + Y_{i+1,k-1} \right] \left[ 1 + j \omega \tau_{a} / Y_{i+1,k-1} \right] \quad k = 1 \text{ to } N - 1
\]

where \( N \) is the total number of compartments. The impedance of the equivalent dendrite tree is \( Z_{D}(\omega) = 1/Y_{0...N-1}(\omega) \).

The impedance of the entire neuron observed from the soma \( Z(\omega) \) is the combination of the somatic and dendritic impedances, \( Z_{D}Z_{E} / (Z_{D} + Z_{E}) \). The impedance of the soma \( Z_{E}(\omega) \) is calculated as \( Z_{E}(\omega) = (R_{md}/A_{cl})/ (1 + j \omega \tau_{a}) \), where \( \tau_{a} \) is the time constant of the somatic membrane.

**Simulations with voltage-dependent conductances**

Simulations of neurons with voltage-dependent conductances were also performed in which each compartment was modeled as a parallel resistor–capacitor combination in parallel with an inductive term representing the voltage-dependent conductance. The admittance \( Y_{i}(\omega) = 1/Z_{i}(\omega) \) of each compartment was modeled as

\[
Y_{i}(\omega) = G_{m}(1 + j \omega \tau_{a}) + G_{V}(1 + j \omega \tau_{V})
\]

where \( G_{m} \) and \( \tau_{m} \) are the membrane conductance and time constant, respectively, and \( G_{V} \) and \( \tau_{V} \) are the voltage-dependent conductance and time constant, respectively. The term on the right in Eq. 3 is a linear approximation to a voltage-dependent conductance conventionally described by a Hodgkin–Huxley-type equation. This approximation is valid for small-voltage excursions around a set membrane potential \( K \) (Koch 1984) and with this restriction could be used to represent a current such as \( I_{L} \) for example. Small hyperpolarizations increase activation of \( I_{L} \) at most resting membrane potentials, producing an inward current. With voltage changes that are slow relative to the \( I_{L} \) time constant at resting membrane potential \( \tau_{a} \), the magnitude of this inward current would be the product of \( G_{V} \) and the change in membrane potential. With rapid changes in membrane potential \( \omega > 1/\tau_{a} \), \( I_{L} \) would change less and the right-hand term in Eq. 3 would contribute correspondingly less to the compartmental admittance.

\( G_{m} \) was determined by dividing the membrane area of the compartment by the specific membrane resistivity. When the voltage-dependent conductance was restricted to the soma, impedance functions were calculated as described above. For dendritic locations, the cable equations were replaced with equivalent circuit models, using Eq. 3 to represent the membrane properties of each compartment. Compartmental properties were interconnected with axial resistances, given by the product of \( R_{c} \) and \( l_{c} \) divided by cross-sectional cable area. The impedance function of the dendritic cable was computed by starting at the last compartment, adding axial resistance to \( 1/Y_{i}(\omega) \), then taking the inverse to determine the combined admittance. This process was continued until all dendritic compartments had been incorporated. Impedance functions, based on cable equations and equivalent circuits, were compared to ensure that the different methods yielded the same results.

**Estimation of time constant and electrotonic length**

The system time constant \( \tau \) and electrotonic length of each modeled neuron were determined to examine the dependency of the impedance functions on equivalent parameters. To determine \( \tau \) in neuron models with nonuniform membrane resistivities, effective values of \( \tau \) were estimated from the product of specific membrane capacity and an effective membrane resistivity. This resistivity was the inverse of the sum of the somatic and dendritic conductances, weighted by the relative surface areas of the somatic and dendritic compartments. The resulting estimate \( \tau_{eff} \) was then divided by an empirical correction factor \( K \), which compensates for a systematic underestimation that is tightly correlated \( (r^{2} = 0.98) \) with \( \rho \) (Fleshman et al. 1988). For the somatic shunt model, \( K = 1.00 - 0.01 \times [11.2 - 22.0 \ln(\rho)] \). Electrotonic length \( L \) was defined as the length \( (\text{normalized by } \lambda) \) at which the cumulative surface area of the dendritic cable reached 97% of its total area, a convention based on the finding of Fleshman et al. (1988) that the measured value of \( L \) corresponded to the length at which 96–98% of dendritic surface area was attained.

**Comparison of model properties and experimental observations**

Because the dendritic tree of each motoneuron was simplified to an equivalent cable and the dimensions of our standard cable differed from the equivalent cables of Fleshman et al. (1988) to some degree, we compared the calculated properties of the 6 models to those observed experimentally. Excellent matches were seen for both input resistance and \( \tau \), as indicated by regressions \( (R_{exp} = 1.08 \times R_{model} - 0.17 \text{ MO}, r^{2} = 0.98, \tau_{exp} = 0.95 \times \tau_{model} + 0.04 \text{ ms}, r^{2} = 0.99) \). As an additional check on \( \tau \), the transient response to a 1-mV pulse was calculated by multiplying the impedance function times the fast Fourier transform (FFT) of the pulse, and performing the inverse FFT on the product. A model transient computed in this way is shown in Fig. 1C with the experimental transient, plotted as open circles. The agreement between experimental time constants and those determined from model transients (fit between 30 and 35 ms of the transient tail) was also good \( (\tau_{exp} = 0.95 \times \tau_{model} + 0.18 \text{ ms}, r^{2} = 0.98) \).

In summary, errors introduced as a result of the simplifications inherent in the model are not large. The equivalent cable models described herein should be adequate.

**Simulation of changes in the impedance function during synaptic activation**

To model the effects of synaptic input, the conductivity of the affected compartments was increased by dividing the membrane resistivity by the factor \( 1 + x \), where \( x \) represents the fractional increase in conductance attributed to the synapse; for example, to produce a 25% increase in membrane conductance, the membrane resistivity was divided by 1.25. In most simulations, each active synaptic input was represented by an increased conductance in 3 adjacent compartments, a span of 0.15A. This number of compartments was chosen because 0.15A is an approximate mean for the range of locations spanned by the synapses of individual Renshaw cells on the dendrites of motoneurons (Fyffe 1991). In some cases, broader distributions of synaptic input were simulated, as described in Results. Typically, the middle compartment containing active synapses was placed at one of 5 dendritic locations on the model neurons (0.15A, 0.30A, 0.45A, 0.60A, and 0.75A from the soma), and the
membrane conductance was increased by 10, 25, 50, or 100%. This approach implicitly assumes that the probability of synaptic contacts is proportional to the membrane area. In the step model, the total conductance corresponding to a 100% conductance increase ranged from 25.8 to 71.5 nS (mean of 47.3).

In the sigmoidal model, in which resistivity varies by compartment, an alternative approach was used. First, the effective mean $R_{\text{md}}$ of the dendritic cable was determined as the inverse of the sum of all compartment conductivities, weighted by the fractional area of each compartment relative to total dendritic membrane area. Then the membrane area of a 0.15A-long cable segment having this mean $R_{\text{md}}$ was determined. The conductance increase of this cable segment produced by the specified fractional change in conductance (e.g., 25%) was determined, using the same procedure as for the step model. The conductivity of the affected compartments was then increased by the amount needed to match this conductance increase. This approach added the same synaptic conductance at each synaptic site independent of synaptic location, as done implicitly in the step model. The conductance corresponding to a 100% conductance increase ranged from 32.2 to 106.9 nS (mean of 66.6).

The impedance function for the neuron was reevaluated with each change in synaptic input, using the altered resistivities, electrotonic lengths, and time constants of the compartments with synapses.

Simulations of impedance functions obtained from noisy recordings

The effects of noise on estimates of synaptic location and conductance magnitude were assessed using impedance functions of the model neurons to which random Gaussian noise was added. In each trial of the simulations, one of 3 motoneuron models (41/2, 43/5, and 42/4) and one of 3 dendritic locations (0.15A, 0.3A, and 0.45A) were selected randomly using a second random-number generator. A conductance increase of 50% was added to the selected synaptic compartments, spanning 0.15A. The level of added noise was based on the normalized random error for an impedance estimate $E_z(f)$:

$$E_z(f) = \left[1 - \left(\frac{\gamma(f)}{\gamma(\infty)}\right)^{1/2}\right]/\left(2n\gamma(f)\right)^{1/2}$$

where $\gamma(f)$ is the coherence between injected current and the voltage response, and $n$ is the number of samples used to determine the impedance function (Bendat and Piersol 1986). The coherence estimates the fraction of power in the voltage signal produced by the injected current acting on the impedance; that is, a $\gamma(f)$ of 0.99 means that at frequency $f$, 99% of the power spectrum of the membrane voltage is linearly related to the injected current (Bendat and Piersol 1986). Simulations used typical values from the accompanying study of Maltenfort et al. (2004). Coherence was 0.89 at the lowest frequency, 4.88 Hz, increased linearly to 0.98 at 20 Hz, and was constant at higher frequencies. Fifty experimental trials were often taken in this study, and each trial was divided into 5 segments of data (each overlapping the next by 50%) for computation of power and cross spectra and impedance functions. For example, if segment 1 is composed of points 1–1,024, segment 2 would constitute points 513–1,536, segment 3 would constitute points 1,025–2,048, and so forth. This procedure minimized the variance in the spectral estimates, providing effectively 4.1 samples per record (Press et al. 1992), and a total $n$ of 4.1 × 50 = 205. In the simulations, values produced by a Gaussian random-number generator, set to have a SD determined by the preceding equation and the cited values of $\gamma(f)$ and $n$, were added to each point in the impedance functions, with and without synaptic activation. The difference between the 2 impedance functions in each run of the simulation was computed, the reversal frequency was selected, and the change in impedance magnitude was determined. This process was performed without the operator’s knowledge of the model neuron or synaptic location used in the trial. From 11 to 20 trials were collected for each combination of model and synaptic location.

Computations were performed using either programs written in C code or using MATLAB (MathWorks, Natick, MA).

RESULTS

Shape of the impedance function and dependency on electrotonic parameters

MAGNITUDE OF THE IMPEDANCE FUNCTION. Impedance functions calculated for 6 motoneurons modeled with the somatic shunt model using values from the data of Fleshman et al. (1988) are shown in Fig. 1B. Representation of motoneurons modeled with the sigmoidal model and based on the same data of Fleshman et al. (1988) yielded impedance functions that did not differ considerably from results with the somatic shunt model (Fig. 1B). Transient responses calculated from both impedance functions matched experimental responses well (see METHODS). For example, Fig. 1C shows the transient response of a neuron modeled with the somatic shunt model to a 1-ms current pulse (parameters based on cell 42/4; Fleshman et al. 1988). An electrophysiologically recorded transient from cell 42/4 is plotted on the model response and shows good agreement (Fig. 1C).

The effect of electrotonic parameters on the form of the impedance functions was explored. Figure 2A shows the effect of system time constant $\tau$ on the normalized impedance function of the model of motoneuron 43/5. Increasing or decreasing $\tau$ by 50% shifts the impedance function proportionally to the left or right, respectively, along the frequency axis. Multiplying frequencies by $\tau$ compensates for variations in time constant, producing impedance functions for neurons with different $\tau$ values that are superimposable (figures not shown). Figure 2B illustrates the dependency of the impedance function on the dendritic-to-somatic conductance ratio $\rho$. Increasing $\rho$ by 50% (by decreasing soma area) shifts the impedance function to the left, whereas decreasing $\rho$ shifts the curve to the right, so that the roll off in the impedance function occurs at higher frequencies. However, the effect of $\rho$ is not a simple proportional shift, given that the curvature in the roll off increases at larger values of $\rho$. The electrotonic length (L) of the dendritic cable also affected the impedance function, although to a lesser degree. Decreasing L produced a slightly faster roll off of impedance with frequency, whereas increasing L had the opposite effect (not shown).

The impedance functions for all 6 models are shown in Fig. 2C. The effect of time constant was removed by normalizing frequency by $\tau$ for each neuron. The differences between these impedance functions are best correlated with differences in $\rho$. The 3 models with $\rho = 0.14–0.32$ show a later and more linear fall-off of impedance with frequency than do the 3 with $\rho = 1.1–1.2$ (whose lines overlie each other in this figure). The 2 impedance functions, which have similar values of $\rho$ (0.3 vs. 0.32), are close together and situated between the impedance curves representing cells with larger and smaller values of $\rho$.

The influence of $\rho$ on the impedance function of neurons with low somatic resistivity can be attributed to the difference in the somatic and dendritic impedance functions and the difference in membrane time constants. The somatic and dendritic contributions to the motoneuron’s impedance function depends on their relative conductance, as indicated by $Z = Z_sZ_d/(Z_s + Z_d) = 1/(Y_s + Y_d)$. The shapes of somatic and
Because R_m and R_md and their associated time constants may vary, the phase of the impedance function differs, as shown for one of the model neurons in Fig. 2A, where the somatic impedance behaves as a simple one-pole low-pass filter, whereas the curvature in dendritic impedance reflects its distributed, cable structure. Because R_m and R_md and their associated time constants may differ by 2 orders of magnitude, somatic and dendritic impedance functions roll off at very different frequencies, with dendritic impedance decreasing over a broad frequency range where somatic impedance remains nearly constant. Within this range, dendritic conductance increases and dendritic characteristics dominate the impedance function, as indicated by the relation. This effect is greater with larger values of ρ.

**PHASE OF THE IMPEDANCE FUNCTION.** Phases of the impedance function for all 6 model motoneurons are shown in Fig. 3A. Similar to impedance magnitude, the impedance phase of the sigmoidal and step models did not differ significantly. Some of the variability between cells is accounted for by differences in τ. Changes in τ affect phase in the same manner as they affected the magnitude of the impedance function. Phase curves of models that differed only in their values of τ could be superimposed after normalization by τ (not illustrated). The effects of L and ρ were more complex. Changing L produced changes in curvature of the phase relation, such that greater curvature was observed as L decreased, and less was observed as L increased (Fig. 3B).

Nelson and Lux (1970) demonstrated the sensitivity of phase to ρ, based on modeling work of Rall (1960). In our simulations, we found that as ρ varied, the curvature of the phase plot changed, but the frequency at which the phase lag was 45° was constant, equal to 1/(2πτ,), where τ was the somatic time constant. Because the dendritic membrane has a much longer time constant than τ, (Clements and Redman 1989; Fleshman et al. 1988), the dendritic impedance phase reaches a maximum of 45° at relatively low frequencies, and τ determines the

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**FIG. 2.** Effect of τ, ρ, and L on impedance functions. A: impedance functions are plotted for model motoneuron 43/5 for 3 different values of system time constant τ. Normal value of τ for this cell is 7.6 (long dashed line); this value was increased by 50% (solid line) or decreased by 50% (dashed line) by changing specific membrane capacitance. B: in this plot, the dendritic-to-somatic conductance ratio (ρ) was changed for the same motoneuron model by changing somatic area. Solid line indicates an increase of ρ by 50% and the short dashed line, a decrease by 50% from the normal value of 1.2. C: this figure shows the impedance functions of the 6 model motoneurons illustrated in Fig. 1 plotted against normalized frequency (frequency × τ). Same key is used as in that figure. D: impedance functions of the soma and dendritic cable of model motoneuron 36/4 are plotted in this figure. All impedance functions are normalized by input resistance. Somatic shunt model was used to represent motoneurons with nonuniform membrane resistivity in this and subsequent figures, except where noted.

**FIG. 3.** Dependency of the phase of the impedance function on L and ρ. A: plots of phase are shown for the 6 model motoneurons. B: effect of electrotonic length of the dendritic cable (L) on the phase of motoneuron model 43/5 are shown. L was increased by decreasing dendritic diameter (solid line) and decreased by increasing diameter (dotted line). Somatic area was changed to maintain ρ constant. C: effect of changing ρ on the phase of the same motoneuron is represented in B. Phase is shown for the measured value of ρ, for half the measured value (short-dashed line), and twice the measured value (solid line). D: phase is plotted for 3 values of ρ as in C, but the model has been adjusted to fit the assumption that somatic and dendritic resistivities are equal (31% overestimate in resistance; see Fleshman et al. 1988, their Table 1). Variations in ρ affect the phase angle of the impedance function at higher frequencies than variations in L. Accordingly, the frequency axes in C and D differ from the axes in A and B.
frequency at which the neuron impedance phase is 45°. Rall (1960) showed that phase shifted as a function of p without the crossover shown in Fig. 3C, assuming uniform membrane resistivity. When the phase plots were recalculated making that same assumption (Fig. 3D), we obtained a similar result.

Changes in impedance function with tonic synaptic input

DEPEN DENCY OF THE CHANGE IN THE IMPEDANCE FUNCTION ON LOCATION AND MAGNITUDE OF THE SYNAPTIC CONDUCTANCE. Figure 4A compares impedance functions of a neuron with synaptic conductances of 100% at one of 3 dendritic locations (0.15, 0.30, and 0.55λ). This change in conductance totals 34.6 nS in the 3 dendritic compartments. The impedance functions with dendritic synaptic conductances (broken lines) lie between impedance functions of neurons without any synaptic conductance (top solid line) and with a somatic synaptic conductance of the same magnitude (34.6 nS; bottom solid line). The normalized impedance changes are shown in Fig. 4B. Dendritic conductances produce changes in the magnitude of the impedance function that decrease rapidly with frequency. At greater frequencies, an increase in impedance magnitude is actually observed, in agreement with Fox (1985).

The frequency at which the change in impedance reverses sign, which we will call the reversal frequency (F_r) increases as synaptic location moves closer to the soma. In the simulations represented in Fig. 5A, F_r varies inversely with the electrotonic distance of the synapse from the soma. F_r at each synaptic location was determined for several values of conductance change, plotted with overlapping symbols. Varying conductance magnitude had little effect on the value of F_r but did affect the normalized change in input resistance (\%ΔR), as shown in Fig. 5B. \%ΔR increases linearly with the magnitude of the conductance change at each synaptic location.

Such observations suggest that F_r and \%ΔR can provide estimates of synaptic location and conductance magnitude, respectively. However, it is obvious from Fig. 4A that dendritic conductances of this magnitude produce small impedance changes measured at the soma (cf. Carlen and Durand 1981; Rall 1967). To explore the sensitivity of F_r and \%ΔR estimates to noise in the impedance records, a set of simulations was performed in which noise was added to the impedance functions of 3 of the model motoneurons with and without a synaptic conductance of 50%. The added noise was comparable to the level observed in the accompanying study by Malt- enfort et al. (2004). Several alternative methods of measuring reversal frequency and measures of the impedance change at low frequencies were assessed in these simulations. F_r could be identified most reliably by visual identification after passing the impedance magnitude function through a median filter: each point was replaced by the median value of points in a window (ranging from ±3 to 10 points) around the original point.

The variability in \%ΔR was deemed unacceptable, and an alternative measure of the low-frequency impedance change was adopted. This measure, cuΔZ, is a normalized, frequency-weighted cumulative sum, which approximates the average value of ΔZ in a semilogarithmic plot

\[
cuΔZ = \sum (ΔZΔf/f)/\sum Δf/f
\]  

In Eq. 5 ΔZ is the normalized change in impedance magnitude, \(100 \times (|Z(f)| - |Z_{syn}(f)|)/Z(0)\); Δf/f is the frequency interval divided by the frequency of each term in the summation (approximating ln (f)); and the summation is taken from the lowest frequency (4.88 Hz in these studies) in the spectrum to F_r. Division by \(\sum Δf/f\) reduces the dependency of cuΔZ on F_r. CuΔZ is plotted as a function of synaptic conductance for several dendritic locations in Fig. 5C, showing that this measure is proportional to the synaptic conductance change.

The means and SDs of cuΔZ and F_r for sets of measurements made with noisy impedance functions from 3 motoneuron models are shown in Fig. 6, A and B, respectively. Figure 6A indicates the level of uncertainty to be expected in estimates of cuΔZ and its variation between cells. The least variability in relation to the expected cuΔZ value occurs with model 43/5, in
which $\rho$ is largest and the relative change in impedance greatest. The greatest variability occurs with model 41/2, which has the lowest value of $H_{9267}$. The corresponding variability in $F_r$ is shown in Fig. 6B, in which $F_r$ estimates are plotted against the ratio of the mean to SD of the $cu\Delta Z$ estimate. At the larger values of this ratio, above 1–1.5, reasonably accurate $F_r$ estimates can be obtained. These observations suggest a strategy for experimental implementation: determine the expected SD of $cu\Delta Z$ using Eq. 4 and reject $F_r$ estimates if the expected SD is too large in relation to the $cu\Delta Z$ estimate. This approach is applied in the accompanying paper (Maltenfort et al. 2004).

Figure 7, A and B illustrate the dependency of $F_r$ and $cu\Delta Z$ on the magnitude and position of conductance changes for 2 of the model neurons. In these grids, specification of the reversal frequency provides an estimate of synaptic location that is scarcely influenced by conductance magnitude. Once synaptic location is specified, the value of $cu\Delta Z$ estimates the relative change in conductance in the dendritic compartments with active synapses. The differences in these 2 diagrams also show that the relationships between $F_r$, $cu\Delta Z$, and synaptic location and conductance magnitude vary between cells, evidently depending on the electrotonic parameters of each motoneuron.

**FIG. 5.** Effects of electrotonic synaptic location and conductance magnitude on reversal frequency and normalized impedance change. A: inverse of reversal frequency is plotted vs. mean electrotonic position of the active synapse for different values of conductance change. Reversal frequency is the frequency at which the change in impedance reverses sign from a decrease to an increase, as shown in Fig. 4. Values of the conductance change were 10, 25, 50, and 100% of the resting conductance in the dendritic compartments containing the synapses. B: $\% \Delta R$, the normalized change in input resistance [i.e., $100 \times \Delta Z(0)/Z(0)$], is plotted vs. conductance change for different values of mean electrotonic position of the active synapses. Locations of the synapses for each set of simulated points are indicated to the right of the figure. C: an alternative measure of the impedance change at low frequencies, which is less sensitive to noise, $cu\Delta Z$, is plotted vs. conductance (see text); $cu\Delta Z$ values at 0.3$\lambda$ were similar to those at 0.15$\lambda$ and omitted for clarity. Model was based on neuron 43/5 from Fleshman et al. (1988).

**FIG. 6.** Effect of noise on estimations of $F_r$ and $cu\Delta Z$. Simulations were performed in which noise was added to 3 model motoneurons, with synaptic conductances at 3 dendritic locations. Conductance changes were 50%. A: estimates of $cu\Delta Z$ obtained from multiple trials with each combination of model and synaptic location are plotted. Each mean is indicated by a symbol and the associated SD is a vertical line. Synaptic locations and models are indicated in the figure. Each horizontal line marks the expected value of $cu\Delta Z$. B: mean $\pm$ SD of $F_r$ estimates are plotted as a function of the ratio of the mean value of $cu\Delta Z$ to its SD. Different symbols correspond to the model used for each set of values, as indicated in A. Horizontal lines give expected values of $F_r$. 

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FIG. 7. Changes in reversal frequency and cuΔZ with synaptic location and conductance magnitude. These grids show the loci of reversal frequencies and normalized resistance changes for a range of synaptic locations and conductance magnitudes. A and B: step models of an FF motoneuron and an S motoneuron, respectively [motoneurons 38/2 and 36/4 from Fleshman et al. (1988)]. Each set of points connected by near-vertical lines represents the values of reversal frequency and cuΔZ obtained by activation of synapses at a single mean location, as indicated by the numbers at the top of each line. Lines running transversely connect points produced by the same synaptic conductance, as indicated by the numbers along the right side. Conductance values signify the relative change in conductance across 3 contiguous dendritic compartments, each with an electrotonic length of 0.05. Grids based on sigmoidal models of the same neurons are shown in C and D, whereas E and F depict grids based on models with uniform Rm (i.e., without a somatic shunt). Relative conductance changes in the sigmoidal model were based on the area-weighted average of dendritic conductivity. These conductance changes were adjusted for differences in compartment area so that the same total conductance change was applied at each synaptic site. Decline in cuΔZ at more distant synaptic location in the step and uniform models largely reflects a decrease in area of compartments with synapses.
Figure 7, C–F show that the same approach can be used for other motoneuron models. The grids in these figures are based on the same motoneurons as in Fig. 7, A and B, but those in Fig. 7, C and D are based on sigmoidal models, whereas those in Fig. 7, E and F are based on models without somatic shunts, in which somatic resistivity is the same as the dendritic resistivity of the corresponding step models. Reversal frequencies in the sigmoidal grids are considerably larger for proximal locations, and cuΔZ values attenuate less at distal locations than in the step-model grids because of the different electrotone profiles of the 2 models.

**Dependency of Changes in Impedance on ′τ′ and ′ρ′**  
Fox (1985) reported that impedance functions were invariant with ′τ′ when plotted as a function of normalized frequency (i.e., frequency × ′τ′). Considering the importance of relative somatic and dendritic conductances in determining impedance (see above), ′ρ′ should be an important determinant of cuΔZ. That is, dendritic conductance changes will have a greater effect on impedance in neurons with larger relative dendritic conductance (larger ′ρ′). We examined the dependency of ′F_r′ and cuΔZ on ′τ′ and ′ρ′ in the limited set of 6 model motoneurons by comparing parameters in pairs of neurons. When reversal frequencies of each motoneuron for a set of synaptic locations were compared with the corresponding values of ′F_r′ for the other 5 motoneurons, strong linear relations were found (r² > 0.99). A similar finding was made when values of cuΔZ for each motoneuron, obtained for multiple synaptic locations and conductance magnitudes, were compared with the corresponding cuΔZ values of other motoneurons (r² > 0.99). Regression slopes varied between cell pairs.

The dependency of ′F_r′ on ′τ′ was examined by comparing the slopes of the regressions between ′F_r′ values with the ratios of the system time constants of each pair of cells. If ′F_r′ is linearly proportional to 1/′τ′, as suggested by Fox (1985), then the regression slope should equal the inverse ratio of time constants. Regression lines were calculated between reversal frequencies for each cell pair. The slopes of these regressions matched the inverse ratios of time constants of the 2 neurons (i.e., ′F_r1/F_r2 = τ_f1/τ_f2′; r² = 0.96), indicating that reversal frequency is proportional to 1/′τ′. A similar analysis was performed to examine the relationship between cuΔZ and ′ρ′. In this case, cuΔZ was found to depend on both ′τ′ and ′ρ′. This relation (r² = 0.96) was described by: cuΔZ_cuΔZ₂ = (ρ₁/ρ₂)⁰.⁴⁶(τ_f₁/τ_f₂)⁰.³³.

The use of ′ρ′ and ′τ′ to adjust the estimates of synaptic conductance magnitude and location is illustrated in Fig. 8. In Fig. 8A, the grids have been normalized by multiplying ′F_r′ values by ′τ′ and multiplying cuΔZ values by ′τ′⁻¹′ρ′⁻⁰.⁴⁶. This rescaling causes a substantial, if not exact, overlap. Normalizations were also applied to sigmoidal and no-shunt models, illustrated in Fig. 8, B and C, based on analyses such as those described for the step model. CuΔZ in the sigmoidal model was proportional to ′F_r′⁻¹′ρ′⁻⁰.⁴⁶ (r² = 0.91), and normalization by this factor removed most of the variance in cuΔZ values between neurons. However, the correlation between ′F_r′ and 1/′τ′ was weaker (r² = 0.56), as evident in the difference in ′F_r′ values of the normalized grids in Fig. 8B. The failure of ′τ′ to normalize ′F_r′ in this model can be attributed to the nonuniformity of ′R_m′ and membrane time constant. Differences in ′F_r′ values for conductances at 0.15λ, for example, were associated with differences in membrane ′τ′ within this section of dendritic cable.

With the small somatic conductance of the no-shunt models, ′ρ′ is not a significant factor. The relatively small variance in cuΔZ values (slopes of cuΔZ regressions ranged from 0.85 to 1.17) depended on dendritic geometry, specifically ′D_eq′ and on

**Figure 8.** Normalization of impedance grids using ′τ′ and ′ρ′. This figure shows grids after normalization of reversal frequency and cuΔZ with ′τ′, ′ρ′, and ′D_eq′. Neuron models represented are the same as in Fig. 7. Models representing motoneuron 38/2 are indicated by the thicker lines. Reversal frequency was normalized by multiplication by ′τ′ (in s) for all 3 motoneuron models. For the uniform-′R_m′ model, cuΔZ was normalized by multiplication by ′τ′⁻¹′ρ′⁻⁰.⁴⁶ and ′τ′⁻¹′ρ′⁻⁰.⁴⁶(′D_eq/′D_m′)⁻⁰.²³, where ′D_m′ is the mean initial diameter of the dendritic cable for the 3 models (35 μm).
what lower for a given cable position. Consequently, without adjustment for electrotomnic parameters, $F_r$ can be used to estimate the spatial location of synapses along an equivalent dendritic cable, with little dependency on the underlying model assumptions.

DEPENDENCY ON DISTRIBUTION OF SYNAPSES. All simulations described to this point were based on a conductance distributed equally over 3 dendritic compartments (0.15A). A specific synaptic input may produce conductance changes over a range of electrotomnic distances (Burke and Glenn 1996; Fyffe 1991), so we examined the effect of changing the width of the synaptic distribution.

Figure 10A shows that increasing the width of the distribution to cover longer dendritic segments moves the relation between $F_r$ and mean synaptic location upward and to the right. This effect seems to be produced by the more proximal synapses in the wider distribution, which have a greater effect on $F_r$. Despite this proximal bias, plots of $F_r$ versus electrotomnic location of the most proximal synapse in the distributions demonstrated a greater dependency on distribution width (not illustrated), indicating that $F_r$ is a better estimator of mean synaptic location.

The shape of the distribution is also a factor. Less sensitivity to the width of the distribution is seen when the synapses are proximally weighted (Fig. 10B), so that synaptic density is maximum at the proximal edge of the distribution and falls to zero at the distal edge (comparable to Ia inputs; Burke and Glenn 1996). Half the total conductance change occurs within the proximal 29.3% of the proximally weighted distribution, accounting for the smaller influence of width on $F_r$ and the larger values of $F_r$ for a given mean synaptic location (compare Fig. 10A and 10B). $F_r$ was even less dependent on the width of triangular distributions (not shown), in which synaptic density is maximum at the center of the distribution and falls linearly to zero at either end. Allowing for variations in the width and shape of synaptic distributions, $F_r$ estimates electrotomnic location within 0.2 length constants of its true value.

Simulations were also performed to examine the use of $F_r$ to determine the location of synapses confined to part of a dendritic arbor. Models were constructed with 2 dendritic cables. The ratios of the initial diameters of the 2 cables ranged from 0.66 to 1, and the ratios of their electrotomnic lengths (at 97% dendritic area) ranged from 0.66 to 1. The profiles of the

$\tau$, with $\tau$Z proportional to $D_{eq}^{0.23} \tau^{0.45}$ ($r^2 = 0.95$). The $\tau$Z differences between normalized grids seen in Fig. 8C are among the worst among the normalized no-shunt grids. $F_r$ values of the grids represented in Fig. 7, E and F (shown normalized in Fig. 8C) are similar, but $\tau$ normalized $F_r$ for other grids with dissimilar values, corresponding to the observation that $F_r$ was proportional to 1/$\tau$ ($r^2 = 0.97$) in this model.

Comparisons were also made between $F_r$ values in relation to mean spatial synaptic location (rather than electrotomnic location) on the equivalent dendritic cable in the 3 models. $F_r$ values of the step and sigmoidal models were essentially the same for corresponding cable positions (Fig. 9). $F_r$ values for models without a somatic shunt were also similar but some-

![](https://www.jn.org/)

![FIG. 9. Reversal frequency is strongly dependent on spatial location of the active synapses on the equivalent dendritic cable, showing approximately the same relation for all neurons and model types. $F_r$ values for the sigmoidal, step, and uniform-Rm models are represented by different symbols, as indicated in the figure. Best fit for both data sets is given by the solid line: reversal frequency (Hz) = 2.78 × position (cm)$^{1.66}$, $r^2 = 0.97$. Best fit for values of the somatic shunt model is given by the dotted line: $F_r = 2.81 × position^{1.63}$, $r^2 = 0.93$. Best fit for values of the sigmoidal model is given by the dash-dot line: $F_r = 2.48 × position^{1.64}$, $r^2 = 0.97$. Values of $F_r$ for the uniform-Rm model were somewhat lower. Best fit for this model was $F_r = 1.89 × position^{1.62}$ ($r^2 = 0.94$).](https://www.jn.org/)

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2 cables were adjusted so that the electrotonic profile of the combined cables (using the 3/2-power law) matched that of the corresponding one-cable model. Fr values for a tonic conductance at a given electrotonic distance from the soma varied as the conductance was placed on either or both cables of these 2-cable models. The SD of Fr values varied from <1 to 6% of mean Fr at different locations. This variability was sufficient to produce some overlap in Fr values of distal locations (>0.45λ), but was generally small. Values of cuΔZ scaled to the total synaptic conductance in each model, taking into account differences produced by different synaptic locations.

**EFFECT OF VOLTAGE-DEPENDENT CONDUCTANCE.** Impedance functions obtained experimentally may show characteristics at low frequencies, indicating the presence of a voltage-dependent conductance (Maltenfort and Hamm 2004; Moore and Christensen 1985; Weckström et al. 1992). This conductance changes both the magnitude and phase of the impedance function, potentially affecting the relationships between synaptic location, conductance magnitude, cuΔZ, and reversal frequency. An additional set of simulations was performed to examine the effect of a voltage-dependent conductance on these relationships. Voltage-dependent conductances were included in somatic or dendritic compartments, or both, by adding an inductive term, Gv/(1 + jωτv), to compartmental admittance (see METHODS). The range of values used in these simulations for Gv and τv, the magnitude and time constant of the voltage-dependent conductance, covered the values found by Maltenfort and Hamm (2004).

Figure 11 shows the influence of a voltage-dependent conductance, distributed uniformly throughout the neuron, on the impedance function of one model motoneuron. Addition of a voltage-dependent conductance (Gv = 100 μS/cm², τv = 20 ms) altered the form of the impedance change produced by a synaptic conductance, as shown in Fig. 11A. The voltage-dependent conductance increased neuron conductance and lowered impedance over a low-frequency range determined by τv (see Maltenfort and Hamm 2004). This conductance increase had opposing effects on the normalized impedance change induced by synaptic activity, shunting the synaptic conductance at low frequencies, but also reducing the low-frequency impedance value in the denominator of the normalization. Provided that τv was short enough that the frequency

![Image](https://via.placeholder.com/150)

**FIG. 11.** Effect of voltage-dependent conductances on reversal frequency and cuΔZ. A: impedance magnitude changes produced by a 100% conductance increase at 0.45λ are shown for a model motoneuron with and without a voltage-dependent conductance with amplitude Gv of 100 μS/cm² and time constant τv of 20 ms. B: changes in Fr and cuΔZ produced by this voltage-dependent conductance are evident in the differences between the impedance grids (thick lines: with Gv; thin lines: without Gv). C: relative change in Fr produced by voltage-dependent values of several magnitudes (50, 100, and 200 μS/cm²) and time constants (5, 20, and 50 ms) are shown. A tonic synaptic conductance of 100% (90.9 μS/cm²) at one of 4 locations, as indicated in the figure, was used in these simulations. D: corresponding relative changes in cuΔZ are shown. Symbols representing different synaptic locations are the same as in C. All simulations were performed with a step model based on motoneuron 43/5 from Fleshman et al. (1988).
range of the voltage-dependent conductance extended to the reversal frequency, $F_r$ was also affected. These effects are evident in the 2 grids shown in Fig. 11B. This voltage-dependent conductance increases $\Delta Z$ values and increases $F_r$, progressively with more distal synaptic locations.

Figure 11, C and D show the effects of different $G_V$ and $\tau_V$ values for a uniformly distributed voltage-dependent conductance. $F_r$ increases as $\tau_V$ decreases, the effect of which was greater at more distal synaptic locations. Except for $\tau_V < 20$ ms and $G_V \geq 100 \mu S/cm^2$, these increases are relatively small. A voltage-dependent conductance decreases the apparent input resistance, tending to increase $\Delta Z$, but the added conductance also reduces the change in impedance produced by a synaptic conductance, tending to decrease $\Delta Z$. Consequently $\Delta Z$ is decreased by voltage-dependent conductances with short time constants, but is increased when the time constant is long. These changes may be substantial. Large voltage-dependent conductances that produce this effect should be evident as a low-frequency dip in a neuron’s impedance function (Maltenfort and Hamn 2004).

The effects of voltage-dependent conductance restricted to dendritic locations were similar to those with uniform distribution (not shown). Voltage-dependent conductances restricted to the soma produced qualitatively similar but smaller effects (not shown). (Somatic $G_V$ values were increased by a factor of 10 in these simulations to produce impedance functions similar to those produced by uniform $G_V$.) Changes in $F_r$ and $\Delta Z$ with somatic $G_V$ were 10 to 25% and 30 to 75%, respectively, of those with uniform $G_V$. Changes with the 2 $G_V$ distributions were more similar in cells with larger $\rho$.

**Discussion**

This study has demonstrated that the change in the impedance function of spinal motoneurons produced by a long-lasting synaptic conductance can be used to identify the location of the conductance, confirming Fox (1985). The impedance functions can also be used to estimate the relative magnitude of the conductance change. In the following discussion, we address the relation of this study to previous work that concerned the determination of synaptic conductance changes in neurons, the basis of the impedance change, and the experimental applicability of the impedance method.

**Comparison to previous studies**

The technique described in this report complements several approaches for determining synaptic location and/or conductance. Analysis of postsynaptic potential shape has been particularly effective in estimating the location of synapses producing individual postsynaptic potentials (PSPs; e.g., Iansek and Redman 1973; Jack et al. 1971; Rall et al. 1967; Redman and Walmsley 1983). Unlike the present method, analysis of PSP shape requires a brief conductance change, or an estimate of conductance time course. Estimates of relative location for inhibitory synapses can be obtained by comparing the sensitivity of inhibitory PSPs (IPSPs) to reversal by chloride injection and hyperpolarization (e.g., Burke et al. 1971). Smith et al. (1967) applied impedance methods to determine the time course and magnitude of conductance changes produced by several synaptic systems in spinal motoneurons. However, interpretation of their results was limited by use of a single frequency, which can yield seemingly paradoxical results, as discussed by Fox (1985). More recently, Häusser and Roth (1997) described a method for determining the time course of transient synaptic conductances based on changes in the current measured following step changes in the holding potential of a somatic voltage clamp. Conductance magnitude can be determined with this method if the reversal potential of the postsynaptic potential is known, and if the voltage escape associated with the synaptic current is relatively small. It should also be possible to estimate electrotonic distance of the synapses from the soma using measurements obtained with this method. Under suitable experimental conditions, this method addresses the needs of applications that require characterization of a transient synaptic conductance.

Carlen and Durand (1981) simulated the responses of compartmental models to brief current pulses during tonic dendritic conductance changes. Dendritic shunts decreased both input resistance and $\tau$, but only input resistance was sensitive to shunt location. The principal indication of shunt location was given by how quickly the voltage transient of the neuron with the shunt separated from that of the neuron without the shunt. Considering the relation between measurements in the time and frequency domains, these observations appear to be qualitatively consistent with the observations made in this study. These investigators proposed that the location and magnitude of a tonic conductance change could be estimated by comparing its relative effect on input resistance and $\tau$, and by determining the time of separation between voltage transients from neurons with and without the shunt. Determination of the changes in input resistance and $\tau$ from time domain measurements might provide a useful comparison to the methods discussed in this report.

**Determinants of the reversal frequency**

The increased impedance at higher frequencies resulting from a tonic conductance increase is counterintuitive. Such increases have been observed, however (Fox and Chen 1985; Maltenfort et al. 2004; Smith et al. 1967). Fox (1985) attributed this phenomenon to a smaller phase shift of the voltage response in dendritic regions with a conductance change, resulting in more effective summation of the distributed voltage responses at the soma. As shown in the Appendix, the difference in impedance magnitude is zero whenever the phase of the difference between dendritic admittance functions with and without the conductance increase is 90° greater than the summed phase of the corresponding neuronal admittance functions. Figure 12 shows that the phase difference between dendritic admittances decreases more rapidly for distal synaptic locations, so that reversal frequency occurs at lower frequencies.

The reason for the dependency of reversal frequency on synaptic location is evident when considering the length constant of a uniform dendritic cable as a function of frequency (e.g., Moore et al. 1988).

$$\lambda(\omega) = \left(\frac{R_m \times D}{4R_i}\right)^{0.25} \left(1 + \omega^2 r_m^2\right)^{0.25}$$

where $\tau_m$ is the membrane time constant. Higher frequencies of injected current have shorter space constants and do not effec-
The reversal frequency increases as synapses are positioned closer to the soma. The frequency dependency of the length constant underlies the correspondence between $F_r$ and the spatial location of the synaptic conductance change, which differs little between the somatic shunt and sigmoidal models (Fig. 9). As frequency increases, the electrotonic length at that frequency becomes less dependent on $R_m$ and more dependent on $C_m$. This is evident by rearranging Eq. 6  

$$\lambda(\omega) = \sqrt{[\gamma D/4R_i]} \times (1/R_m + \alpha C_m)$$  

(7) 

Accordingly, there is much less difference between the electrotonic distances at $F_r$ of the somatic shunt and sigmoidal models for a particular synaptic location than between the electrotonic distances based on length constants computed for steady-state potentials. That is, the electrotonic structures of different dendritic representations become increasingly more similar as frequency increases. This finding is illustrated in Fig. 13, in which frequency-dependent electrotonic distances (approximated by application of Eq. 7 to each compartment) are plotted for 2 synaptic locations, 2.0 mm (top lines) and 1.3 mm from the soma (bottom lines), in the somatic shunt (solid line) and sigmoidal models (dotted line). Much less difference exists between the 2 models for electrotonic distance in the vicinity of $F_r$ (marked by stars) than at low frequencies. The lower $F_r$ values of the uniform-$R_m$ model are attributable to the higher $\rho$ of this model, and the smaller contribution of the soma to the neuron’s admittance.

This result suggests that dendritic models are not easily distinguished by tests that use higher frequencies of current

![Image](http://jn.physiology.org/)

**Fig. 12.** Reversal frequency produced by a dendritic conductance change is the frequency at which the phase angle of the change in dendritic admittance ($Y_D - Y_G$) is equal to the phase angle of the summed neuronal admittance functions [with and without the conductance change; $Y_{tot} + Y_{soma}$] plus 90° (see Appendix). Top graph shows normalized changes in impedance magnitude vs. frequency for 3 synaptic locations: 0.15A (thick line), 0.35A (dot-dashed line), and 0.55A (solid line). Zero is indicated by the horizontal dotted line. Bottom graph shows phase shifts vs. frequency of $\angle[Y_{tot} + Y_{soma}] + 90°$ (short dashed line) and $\angle(Y_D - Y_G)$, corresponding to the changes in impedance magnitude shown in the top graph. Curve for $\angle[Y_{tot} + Y_{soma}] + 90°$ actually represents the corresponding 3 summed admittance functions, which were so similar that they could be plotted with a single line. Simulations were based on the somatic shunt model, cell 38/2 from Fleshman et al. (1988), with 100% relative change in conductance in 3 adjacent dendritic compartments.

![Image](http://jn.physiology.org/)

**Fig. 13.** Using the frequency-dependent electrotonic length constant (Eq. 6), electrotonic distances from the soma are plotted as a function of frequency for 2 synaptic locations in the somatic shunt and sigmoidal models. Electrotonic distances are determined by application of Eq. 6 to successive compartments of the dendritic cylinder. Two sets of curves are shown, one representing the electrotonic distance to a synaptic site located at 2.0 mm (top set of lines) from the soma on the equivalent dendritic cylinder, the other at a distance of 1.3 mm (bottom set of lines) from the soma. Each set contains a somatic shunt (solid line) and a sigmoidal (dotted line) representation of motoneuron 38/2 from the data of Fleshman et al. (1988). Asterisks indicate the reversal frequency for each synaptic site in each motoneuron model (1.3 mm location: 78 Hz for the somatic shunt model, 86 Hz for the sigmoidal model; 2.0 mm location: 36 Hz for the somatic shunt model, 37 Hz for the sigmoidal model).
injection, or brief synaptic conductance changes. An earlier modeling effort by Segev et al. (1990) demonstrated that transient conductances placed in the same spatial location produced identical excitatory PSPs (EPSPs) in somatic shunt and sigmoidal representations of a motoneuron. Despite very different electrotonic structures for steady-state currents, these 2 motoneuron models yield similar \( F_r \) values and synaptic potentials for conductance changes at the same dendritic site.

**Applicability to experimental studies**

The average total synaptic conductance produced by a 100% increase in resting conductance in the 6 step models used in this study was 47 nS. For comparison, the average peak conductances of unitary Ia EPSPs and Ia reciprocal IPSPs are 5 and 9.1 nS, respectively (Finkel and Redman 1983; Stuart and Redman 1990). Assuming that single interneurons produce transient conductance changes that obey alpha functions and have the peak conductance \( \xi_{\text{syn}} = 9.1 \text{nS} \) and time-to-peak \( t_{\text{peak}} = 0.4 \text{ms} \) of unitary reciprocal IPSPs (Stuart and Redman 1990), 95 interneurons discharging at a frequency \( f \) of 50 Hz would be required to produce a time-averaged conductance, \( \xi_{\text{syn}} \) of 47 nS \( [n = \xi_{\text{syn}}/(e \times t_{\text{peak}} \times \xi_{\text{peak}} \times f)]; \) Bernander et al. 1991). Substantially fewer Renshaw cells would be required for this conductance, based on estimates of their synaptic conductances (Maltenfort et al. 2004). Thus the conductance values used in our simulations cover the range of physiological interest, as indicated by numbers of identified spinal interneurons (100 Renshaw cells/mm of spinal cord length; Carr et al. 1998) and the estimated conductance produced by tonic composite Ia reciprocal inhibition (175 nS; Heckman and Binder 1991). The corresponding changes in impedance are small, with \( \text{cu}\Delta Z \) values of <5% for 200% conductance changes. As shown in Fig. 6 for smaller conductance changes (50%), considerable error is present in \( \text{cu}\Delta Z \) estimates, and, depending on the size of this error in relation to the \( \text{cu}\Delta Z \) value, in the \( F_r \) estimates. For a given level of coherence between the injected current and voltage signals, error can be minimized by increasing the number of samples (see METHODS). Estimates should be made of this error and data qualified on this basis, with rejection of \( F_r \) values in which the ratio of \( \text{cu}\Delta Z \) value to \( \text{cu}\Delta Z \) error is smaller than 1–1.5. Use of this approach to determine the synaptic conductances produced by recurrent inhibition (Maltenfort et al. 2004) provides excellent agreement with observed locations of Renshaw synapses on motoneurons (Fyffe 1991).

The responses of neuron models with single equivalent cables that approximate dendritic tapering and uneven dendritic lengths are quite similar to those of fully branched models (Clements and Redman 1989; Fleshman et al. 1988; Holmes and Rall 1992). However, there are differences. Fleshman et al. (1988) found that \( \tau_{\text{r}} \) estimates obtained using equivalent cable models were smaller than estimates from fully branched models, providing smaller values of L based on its estimation from the ratio \( \tau_{\text{r}}/\tau_{\text{f}} \). Substantial differences exist between estimated \( \tau_{\text{r}} \) values and the first voltage-clamp time constant between motoneuron models with full and reduced morphology (Holmes and Rall 1992). Holmes et al. (1992) noted that models with multiple cables or full branching tend to overestimate \( \tau_{\text{r}} \) and L because of current redistribution between dendrites of unequal length. Despite the differences that may occur between a fully branched neuron and a model with an equivalent cable, Holmes and Rall (1992) found good agreement between parameters estimated for 2 models of a neuron with known morphology, one with a simplified representation similar to that used in this study and one with a complete representation of the morphology. For this reason, we think that conclusions drawn from use of these simplified models are applicable experimentally to spinal motoneurons with their complex pattern of dendritic branching.

The use of equivalent cable models makes the implicit assumption that the relevant synapses occur on all dendritic branches. However, individual species of synaptic input may not be distributed uniformly through the dendritic tree of a motoneuron (Burke and Glenn 1996; Rose et al. 1995), and regional conductance increases in multiple-cable motoneuron models can provide misleading estimates of electrotonic properties obtained from voltage transients (Rose and Dagum 1988). Our simulations to examine this issue were limited in scope, but indicate that changes in \( F_r \) are small when a synaptic conductance is confined to part of the dendritic arbor, the greatest relative variability occurring with distal synaptic locations. Thus impedance functions should provide reasonable estimates of synaptic location for inputs that occupy only part of the dendritic tree. Estimates of conductance magnitude should be interpreted with respect to the observation that \( \text{cu}\Delta Z \) varies with both the local synaptic conductance change and the area of membrane affected.

Our simulations demonstrate that \( F_r \) and \( \text{cu}\Delta Z \) estimate synaptic location and conductance in 3 different motoneuron models, although the relations between these variables differ between models and, within models, on electrotonic parameters. Use of the step or sigmoidal model is appropriate for recordings with sharp electrodes, although it is unclear which model better represents spinal motoneurons. Without resolving this problem, both models can be used to determine ranges for the estimated synaptic parameters. Assuming that much of the nonuniformity in \( R_m \) in these models reflects injury from electrode penetration, a uniform-\( R_m \) model would be the appropriate choice for data obtained from whole cell recordings, and impedance methods would appear to work well for identifying synaptic location and magnitude. For each model, availability of electrotonic and/or morphological parameters increases estimate accuracy, as shown by the normalizations in Fig. 8. Estimates of \( \tau \) and \( \rho \) can be made with electrophysiological methods, including derivation from impedance functions (Maltenfort et al. 2004), although these estimates are sensitive to departures from assumed electrotonic structure and dendritic organization (e.g., Rose and Dagum 1988). Without the shunting effects of low somatic \( R_m \), variation in the \( \text{cu}\Delta Z \)-conductance relation is smaller in the uniform-\( R_m \) model and insensitive to \( \rho \). The dependency of this relation on \( D_{\text{eq}} \) in this model suggests that dendritic parameters are important in accounting for residual variation not attributable to \( \rho \) in the step and sigmoidal models. An alternative approach to the use of the empirically derived grids is the use of individual model fits for each neurons. Impedance functions can be used to estimate the parameters of each motoneuron required for such models (Maltenfort and Hamm 2004). Even without such models, the relative locations of different synaptic inputs can be assessed by comparing their reversal frequencies.
Voltage-dependent conductances activated at resting potential also affect $F_s$ and $cu\Delta Z$ (Fig. 11). In many motoneurons with evidence of an activated voltage-dependent conductance, the effect of this conductance on $F_s$ and $cu\Delta Z$ is relatively small, judging from estimated values of $G_v$ and $\tau_v$ (Maltenfort and Hamm 2004a). However, with larger conductances, particularly with shorter $\tau_v$ values, the effect on these variables can be considerable. If parameters of the voltage-dependent conductance can be made, the use of individual models that incorporate a voltage-dependent conductance term is one means of addressing this problem. A potentially more serious concern is the possibility that a voltage-dependent conductance would be activated (or inactivated) by the synaptic input under investigation. This complication may induce changes in the $\Delta Z$ function that significantly alter the impedance change produced by a synaptic conductance. For this reason limiting the amplitude of the synaptic potential is important to minimize the change in membrane potential. Furthermore, performing checks on estimates of the synaptic parameters, such as comparing the amplitude of the synaptic potential with the potential predicted by the estimates, is advisable.

One limitation of this method is the need for tonic or long-lasting conductance changes. For a synaptic input, tonic conductance changes may be approximated by stimulation of an input system at suitably high frequencies. For some synaptic systems, such as Ia monosynaptic projections to motoneurons (cf. Finkel and Redman 1983), the conductance change produced by individual stimuli may be so brief that repetitive stimulation cannot be applied at rates fast enough to approximate a constant conductance change. In other cases, the characteristics of synaptic response may be frequency dependent; for example, Heckman et al. (1994) found that repetitive stimulation of cutaneous nerves resulted in a reversal of the postsynaptic potential compared with the response to single stimuli. In the case of recurrent inhibition in spinal motoneurons, the amplitude of inhibition decreases with continued stimulation (Lindsay and Binder 1991; Maltenfort et al. 2004). However, an amplitude decrease can be taken into consideration when interpreting the results of an impedance analysis.

The locations and magnitudes of excitatory and inhibitory synaptic conductances and intrinsic voltage-dependent conductances play a critical role in their interaction and the transformation of synaptic inputs into neuron discharge (e.g., Bernander et al. 1994; Masukawa and Prince 1984; Williams and Stuart 2003). Recordings from large dendrites of cortical and hippocampal neurons in slice (e.g., Magee and Cook 2000; Williams and Stuart 2002) and somatic recordings in slice with the local application of neurotransmitters (e.g., Cash and Yuste 1999; Skydsgaard and Hounsgaard 1994) have provided important insights into dendritic function. However, many neurons do not have dendritic trees suitable for direct recordings, and the use of slice preparations limits the choice of neuron species, developmental stages, and functional states. Impedance measurements could be applied to determine the location and magnitude of synaptic and intrinsic conductances using both in vivo and in vitro preparations, at different membrane potentials and in different functional states. This approach would serve as a useful complement to more direct methods. Impedance-derived conductance estimates, combined with computational approaches, could be useful in analyzing dendritic interactions and control of neuron activity. Application of this method to determine the location and magnitude of synaptic conductance produced by Renshaw cells in spinal motoneurons is presented in the accompanying paper (Maltenfort et al. 2004).

**APPENDIX**

Here we determine the condition necessary for a reversal frequency and separate regions of increased and decreased impedance produced by a tonic conductance increase. Let $Y_S$ stand for the admittance (1/complex impedance) of the soma and $Y_D$ for the admittance of the dendrite. The square of the magnitude of the total admittance of the neuron seen at the soma is

$$Y_{tot}^2 = (Y_S + Y_D)(Y_S + Y_D)^* = Y_S Y_D^* + Y_D Y_S^* + Y_D^2 Y_S + Y_S^2$$

(A1)

where the asterisk denotes the complex conjugate.

The above equation can be reduced to

$$Y_{tot}^2 = Y_S Y_D + 2Y_S Y_D + 2Y_S Y_D + 2Y_S Y_D$$

(A2)

where $Y_S$ and $Y_D$ stand for the real parts of the somatic and dendritic admittances, respectively, and $Y_S^*$ and $Y_D^*$ stand for the imaginary parts.

Now let $Y_G$ stand for the admittance of the dendrite with an increased conductance and $Y_{tot(G)}$ stand for the resulting overall admittance magnitude of the neuron

$$Y_{tot}'^2 - Y_{tot(G)}^2 = Y_S Y_D + 2Y_S Y_D + 2Y_S Y_D + 2Y_S Y_D - Y_S Y_D$$

- $2Y_S Y_D - 2Y_S Y_D - Y_S Y_D$

$= 2Y_S (Y_D - Y_G) + 2Y_S (Y_D - Y_G) + 2Y_S (Y_D - Y_G) + Y_D^2 Y_S$

- $Y_S^2 - Y_D^2$


(AJ)

When there is no change in the magnitude of the admittance


(AJ)

which can be rewritten

$$\tan \angle(Y_D - Y_G) = -\cot \angle[Y_{tot} + Y_{tot(G)}]$$

(A4)

or

$$\angle(Y_D - Y_G) = \angle[Y_{tot} + Y_{tot(G)}] + 90^\circ$$

(A5)

where $\angle$ denotes the phase angle of the complex quantity. Equation A5 indicates that the reversal frequency will occur when the vector representation of the difference between the dendritic admittances with and without the synaptic shunt is orthogonal to the resultant of the vector representations of the admittances of the neuron with and without the additional conductance. In this circumstance, the change in the dendritic admittance produced by the conductance change will not affect that magnitude of the total admittance of the neuron. If the phase angle of the difference in the dendritic admittances is more than 90° greater than the resultant, the neuronal admittance will be greater with the additional conductance, whereas if the phase angle is <90° greater than the resultant, the change in dendritic admittance will produce a decrease in neuronal admittance (i.e., the impedance will be greater with the conductance change).

Using the above proof, we can also demonstrate that a somatic conductance change will never produce a reversal frequency. Let $Y_{G soma}$ stand for the somatic admittance with an open synaptic conductance. In the model considered herein, the admittance of the
somatic compartment is defined as \( Y_{s} = G_{S} (1 + j \omega t) \). Assume when the somatic synapse is opened that the steady-state conductance \( G_{S} \) is multiplied by a factor \( k \). The time constant of the soma will then be smaller by a factor of \( k \) as well, so that \( Y_{\text{G soma}} (s) = k \times G_{S} (1 + j \omega t) \). Therefore \( Y_{s} - Y_{\text{G soma}} = (1 - k)G_{S} \). The phase angle of \((Y_{s} - Y_{\text{G soma}})\) will always be 180°, and so the intersection of phase angles described in Eq. A5 can never take place.

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R E F E R E N C E S


