Firing Properties and Electrotone Structure of *Xenopus* Larval Spinal Neurons

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Saint Mieux, Benoit and L. E. Moore. Firing properties and electrotone structure of *Xenopus* larval spinal neurons. J. Neurophysiol. 83: 1366–1380, 2000. Whole cell voltage- and current-clamp measurements were done on intact *Xenopus laevis* larval spinal neurons at developmental stages 42–47. Firing patterns and electrotone properties of putative interneurons from the dorsal and ventral medial regions of the spinal cord at myotome levels 4–6 were measured in isolated spinal cord preparations. Passive electrotone parameters were determined with internal cesium sulfate solutions as well as in the presence of active potassium conductances. Step-clamp stimuli were combined with white-noise frequency domain measurements to determine both linear and nonlinear responses at different membrane potential levels. Comparison of analytic and compartmental dendritic models provided a way to determine the number of compartments needed to describe the dendritic structure. The electrotone structure of putative interneurons was correlated with their firing behavior such that highly accommodating neurons (Type B) had relatively larger dendritic areas and lower electrotonic lengths compared with neurons that showed sustained action potential firing in response to a constant current (Type A). Type A neurons had a wide range of dendritic areas and potassium conductances that were activated at membrane potentials more negative than observed in Type B neurons. The differences in the potassium conductances were in part responsible for a much greater rectification in the steady-state current voltage (I-V curve) of the strongly accommodating neurons compared with repetitively firing cells. The average values of the passive electrotone parameters found for Rall Type A and B neurons were $c_{soma} = 3.3$ and 2.6 pF, $g_{soma} = 187$ and 38 pS, $L = 0.36$ and 0.21, and $A = 3.3$ and 6.5 for soma capacitance, soma conductance, electrotonic length, and the ratio of the dendritic to somatic areas, respectively. Thus these experiments suggest that there is a correlation between the electrotone structure and the excitability properties elicited from the somatic region.

**INTRODUCTION**

The *Xenopus* embryo and larvae have become extremely useful preparations for investigations of locomotor neural networks. The pioneering studies of Roberts and his coworkers (Roberts 1989) have not only identified the few principal neurons of the network with their synaptic connections but also introduced minimal neural network models that simulate an impressive amount of behavior observed in this preparation (Dale 1995b; Roberts and Tunstall 1990; Roberts et al. 1995). Furthermore whole cell voltage-clamp measurements have been done on cultured (O’Dowd et al. 1988) or isolated cells (Dale 1991, 1995a) as well as intact surface neurons (Desarmenien et al. 1993; Prime et al. 1998, 1999). Quantitative models describing these measurements have been restricted to a single somatic compartment (Dale 1995a; Lockery and Spitzer 1988) because dendritic structures are minimal at early developmental stages.

Although the dendritic structure at stage 37/38 is minimal, it clearly exists as shown by both morphology and electrophysiological estimates of the electrotonic structure (Soffe 1990; Van Mier et al. 1985). This is in keeping with the original empiric model of Roberts and Tunstall (1990) that has three compartments representing the dendrite, axon, and the soma. Thus it would appear useful to understand the dendritic properties as they begin to develop especially with regard to the appearance of NMDA receptors (Prime et al. 1999) because they appear to be correlated directly with the presence of dendritic trees (Prime 1994; Prime et al. 1998).

The whole cell clamp experiments reported here were done at larval stages 42–47 where the dendritic structure is clearly more developed (Van Mier et al. 1985) and likely to play a definitive role in locomotor behavior. The major advantages of our approach are the normal electrical activity of functional neurons can be measured as demonstrated by patterned network behavior (Fig. 1), the neurons are not isolated from their normal milieu, and thus minimal distortions in structure are likely to have occurred because of measurement procedures as is demonstrated by the maintenance of synaptic events during the experiments.

In addition, both real-time and frequency domain measurements were used to determine the electrotone behavior and take into account inevitable electrode properties (Moore and Christensen 1985; Wright et al. 1996). These experiments suggest that there is a correlation between the electrotone structure and the excitability properties elicited from the somatic region. The action potentials of the neurons that showed strong accommodation also had different electrotone parameters compared with nonaccommodating neurons. Thus in addition to the effects of the voltage-dependent conductances on firing behavior (Dale and Kuenzi 1997), the structure in which these conductances are expressed is correlated with the rhythmic behavior of the neuron, perhaps as some function of development from the embryo to larval stages.

**METHODS**

**Experimental preparation**

The developmental stages 42–47 of *Xenopus* larvae were obtained after hormonally induced fertilization. Embryos and larvae were de-
veloped at room temperature and the stages were morphologically selected as described by NieuwenKoop and Faber (1994) and Van Mier et al. (1985). In accordance with the European Communities Council directive of November 24, 1986 and following the procedures issued by the French Ministère de l’Agriculture, the larvae were anesthetized in tricaine methanesulfate (MS222 Sigma) and placed in Ringer solution [composed of (in mM) 110 NaCl, 3 KCl, 1.0 MgCl2, 1.0 CaCl2, and 10 HEPES; pH 5.74] containing 0.5 mg/ml dispase (Boehringer, Mannheim). The notochord, spinal cord, and overlying musculature were dissected, and the preparation was agitated for 30–35 min at room temperature, after which the spinal cord was removed easily from all surrounding tissues. Figure 1 illustrates rhythmic locomotor or bursting behavior (Fig. 1A) of an isolated, stage 37–38, spinal cord similar to that observed in the intact preparation (Roberts 1989). Similarly, isolated larval spinal cords (Fig. 1, C and D) show network patterns like those observed in the intact preparation (Fig. 1B). Figure 1D also illustrates bursting activity induced by 50 μM NMDA recorded with suction electrodes.

At the larval stage of development, the connective tissues eventually making up the meninges had to be removed before a patch electrode seal was possible. Although some recordings were made from neurons on the external surface (Desarmenien et al. 1993), the success rate was improved considerably by further dissection to reach the inner regions of the spinal cord and to visualize the neurons. The cells of the spinal cord cut between the otic capsule and the 10th myotome were exposed for intracellular recording by carefully splitting at the midline and mounting the half cord with the inner face up (Fig. 2), revealing groups of neurons: dorsal sensory neurons, presumed medial interneurons, and presumed ventral motoneurons. The positioning of the half cord was done with two micromanipulators attached with suction electrodes or sharp glass rods. All recordings were done from neurons between the 4th and 6th myotome levels. The Rohon-Beard sensory cells (Spitzer 1982) were identified easily on the dorsal surface. Longitudinally lined up large motoneurons in the ventral part of the cord could be distinguished; however, other less visible motoneurons could be confused with presumed interneurons. Intermediate neurons near the inner surface of the half cord in dorsal and ventral medial positions were provisionally identified as interneurons and selected for analysis from a total of 200 recorded neurons of all groups. Twenty presumed interneurons, which showed stable recordings for a minimum of 30 min, were fully analyzed.

**FIG. 1.** Rhythmic activity of isolated Xenopus spinal cords. A: extracellular recording of bilateral activity from an isolated Xenopus embryo spinal cord (stage 37/38) using suction electrodes at the rostral and caudal ends. Rhythmic activity was evoked by a 1-ms pulse of current. Combined frequency is ~40 Hz; however, an alternation of large and small spikes at 20 Hz can be seen in part of the record, suggesting a fictive locomotor pattern typical of the intact preparation. B: simultaneous extracellular recordings of rhythmic bursting activity from both sides of an intact stage 46 Xenopus larvae. Alternating bursts of ~1 Hz occurred spontaneously. C: extracellular recording of bilateral activity from an isolated Xenopus larvae whole spinal cord (stage 46) using suction electrodes. Rhythmic activity was evoked by 50 μM N-methyl-D-aspartate (NMDA) in the perfusion fluid. Burst frequency was ~1 Hz. D: extracellular recording of bilateral activity from an isolated Xenopus larvae half spinal cord (stage 46) that was obtained by cutting the spinal cord of C at the midline. Bursting frequency evoked by 50 μM NMDA was nearly the same as observed from the whole spinal cord; however the burst duration decreased two- to threefold. One-second calibration bar applies to B–D.

**FIG. 2.** Photograph of isolated half spinal cord. Half spinal cord was maintained in a open position with 2 sharp glass electrodes. Top and bottom white lines indicate the borders of the dorsal and ventral regions of the cord. Indicated neurons represent presumed interneurons and the motoneurons. Large round cells on the dorsal surface were identified as Rohan Beard sensory neurons. Calibration bar is 50 μm.
Electrophysiological recording

Figure 3 illustrates the combination current or voltage clamp and sum of sines (white noise) method that was used to obtain real-time (Fig. 3, B and C) and frequency (Fig. 3, D and E) domain responses (Moore and Christensen 1983; Moore et al. 1993). The experimental procedures were designed to measure both nonlinear responses evoked by a constant current, \( I(t) \), or voltage clamp in real time, and steady-state linear behavior in the frequency domain for both clamp modes. The stimulus protocol used for the two domains is illustrated in Fig. 3A by a command step representing either a current or voltage, which is followed by a superimposed steady-state small white noise signal that evokes a linear response (Wright et al. 1986). The corresponding transient voltage (Fig. 3B) or current (Fig. 3C) responses are schematic representations of the influence of active conductances.

The data were obtained with an Axoclamp 2B (Axon Instruments, Foster City, CA), filtered at 500 Hz with an 115 db/octave elliptical filter (Krohn-Hite, Model 3900, Avon, MA) and digitized at 12 bits. The values of both the current and the voltage were measured during voltage- and current-clamp measurements. This point is of some importance because the value of the current measured in current clamp was not identical to the command input. The linear responses were analyzed in the frequency domain to obtain magnitude and phase functions of either the impedance or admittance corresponding to current or voltage clamp, respectively. A fast Fourier transform (FFT) of 1,024 points (0.8 s) of the voltage, \( V(t) \), or current, \( I(t) \), response provided the corresponding output functions of frequency, namely \( V(f) \) and \( I(f) \), as well as the stimulus or command input, \( I_f \) or \( V_f \), for current and voltage clamp, respectively. The corresponding impedance, \( Z(f) \), and admittance functions, \( Y(f) \), then were computed as \( Z(f) = V(f) / I(f) \) and \( Y(f) = I(f) / V(f) \).

A step-by-step description of experimental procedure is as follows: 1) 400-ms step constant currents are injected into the soma, immediately followed by superimposed low amplitude white noise for 1 s. The steady-state responses for both the current and the voltage during the last 0.8 s are analyzed in the frequency domain to obtain impedance magnitude and phase functions, namely the output voltage with respect to the input current. 2) Similar voltage-clamp steps then are done for a range of membrane potentials to obtain the admittance magnitude and phase functions, i.e., output current with respect to input voltage. 3) The resulting real-time transient responses and frequency domain functions then are fitted using parameter estimation techniques with an electrotone model having voltage-dependent conductances to obtain quantitative descriptions of each neuron, i.e., a complete neuronal model with its electrotone structure and kinetic behavior at all membrane potentials for both constant current- and voltage-clamp conditions.

In principle, small-signal current- and voltage-clamp measurements should lead to reciprocal frequency domain functions, \( Z(f) = 1/Y(f) \). This property is a striking example of one of the advantages of transform functions, namely that two types of data can be compared independently of a theoretical model. The equivalence of the two functions provides a test of the current- and voltage-clamp instrumentation (Magistretti et al. 1996), where the latter requires stable electronic circuitry to control the membrane potential at all measured frequencies. Current-clamp responses have the advantage that errors from a control amplifier are minimal compared with the voltage clamp and in general tend to be more reliable. For stable conditions, small signal measurements of the two modes should be consistent, independently of any particular model. Thus the linear frequency domain measurements in voltage- and current-clamp modes provide a minimal test of the voltage clamp and should be compared before any comparisons of real-time voltage- and current-clamp responses are meaningful. Because of this equivalence the data always was plotted as impedance functions, however, all voltage-clamp data are actually admittance measurements. A difference in the impedance functions may occur due to changes in the leakage conductance or electrode
Properties; however, the electrotonic parameters, A and L (see definitions in the following text), were required to be constant.

Because the impedance or its reciprocal, the admittance, is a ratio of response and stimulus, the final form of the data were corrected for the effect of the antialiasing filter. However, the real-time responses are not ratios and consequently contain a damped antialiasing filter response. The 500-Hz band was chosen because it provides the best compromise for the determination of electrotonic and voltage-dependent conductance properties. Because the sampling rate was 0.78125 ms, the determination of transient real-time kinetics is limited to a few milliseconds with some rapid ringing because of the sharp antialiasing filters. This limitation is less severe for the frequency domain because a 1-ms relaxation time has a corner frequency of 160 Hz that is in the middle of the frequency band measured. It is for these reasons that the frequency domain is extremely useful for the estimation of the kinetic behavior.

Figure 3, D and E, illustrates typical magnitude and phase functions for passive neurons similar to those obtained in these experiments. The superimposed plots show that the dendrites impose marked inflections on the phase function that are not present with isolated somatic structures. Magnitude functions are less sensitive; however, they do exhibit small inflections as well. The frequency domain functions thus provide an accurate method to determine the electrotonic structure that is essential for the subsequent steps in the analysis of the active membrane properties.

Electrode properties and compensation methods

Electrodes were pulled with a laser heated puller (Sutter P2000, Sutter Instruments, Novato, CA) from 1.5 mm glass (GC150F, Electromedical Insutruments, Pangbourne, UK). The electrodes were filled with either (in mM) 90 K-glucosone, 20 KCl, 2 MgCl2, 10 HEPES, 10 EGTA, 3 ATP, and 0.05 GTP or 55 Cs2SO4, 55 sucrose, 2 MgCl2, 10 HEPES, 10 EGTA, 3 ATP, and 0.05 GTP. In both solutions, the pH was adjusted to 7.4 with 10 mM KOH. Thus the internal perfusion fluids contained either potassium or cesium as the principle internal cation. Because different internal solutions were used no correction for liquid junction potentials was made. This correction is likely to be ~10 mV based on the considerations of previous measurements (Neher 1992).

The electrode impedance was measured at the depth in the solution of the selected neuron and just before making the gigaseal of the patch. Because the electrode impedance is low and not well matched to the Axoclamp headstage, it was measured in series with a parallel RC electronic model that was used to provide a calibration of the method. Measurements and analyses then were done on the combination electrode and electronic model just as with an actual neuron. The electronic model impedance is comparable with measured neurons and provides a method to simulate the contribution of the electrode in the typical recording situation. Figure 4 illustrates that a change of solution levels of 1 mm led to changes in the electrode capacitance of 2 pF. The data and superimposed model fits indicate that real-time data are relatively insensitive to these small changes; however, the differences in the phase function are quite clear, as indicated by C1 and C2 for the phase impedance of Fig. 4C. Under these conditions, the use of the Axoclamp electronic compensation capabilities for the electrode capacitance and series resistance led to phase functions that could not be reliably estimated (Wilson and Park 1989). Capacitance compensation alone was partially effective. Using quartz glass or coating the electrodes with silicone elastomer (Sylgard) reduced the value of the capacitance; however, it was still not possible to achieve adequate compensation. Because these procedures distort the measured data in an uncalibrated manner, no electronic compensation was done, and the electrode was modeled as part of the measurement system. This had the additional advantage that any changes in the series electrode impedance always would be taken into account because each voltage-clamp record contained high-frequency data that are sensitive to electrode properties. Thus fitting recorded data over a reasonably wide frequency range requires accurate electronic parameters. If the electrode increases its resistance, this will become apparent at high frequencies in contrast to most voltage-dependent conductance effects that are more sensitive to lower frequency ranges. This approach is also more exact than traditional series resistance compensation methods, which are always partial because of stability problems in the voltage clamp.

The mean parameter values of the electrode impedance in the solution just before making the seal were $R_e = 10.5 \, \text{M}\Omega$ and $C_e = 5.69 \, \text{pF}$ (Table 1). All measurements were done with the electrode within a few micrometers of the recorded neuron. This procedure avoided errors in the electrode parameters that were due to different solution levels. The range of $C_e$ corresponding to the lowest and the highest levels of solution, 0.1 and 1 mm, was 2 to 6 pF, respectively.

After making the seal and establishing the whole cell clamp recording, the electrode was refitted using a neuronal model in series with the electrode. The new fit included both the electrode and neuron; however, the electrode properties were estimated over the entire range of solution levels. The range of $C_e$ corresponding to the lowest and the highest levels of solution, 0.1 and 1 mm, was 2 to 6 pF, respectively.

**FIG. 4.** Effect of solution level on electrode capacitance. A whole cell electrode tip was placed in the recording chamber with its connection to ground through an electronic model consisting of a parallel resistance, $R_e$, and capacitance, $C_e$. Electrode was modeled as a resistance, $R_e$, and capacitance, $C_e$, as discussed in the Appendix. A: superimposed real-time responses to −10 pA of current were measured and fitted for 2 solution levels. Four curves cannot be distinguished showing that the real-time response is insensitive to the solution level. B: impedance magnitude plots for the same conditions as A. C: phase functions as in B in which differences in the responses and fitted curves can be seen at high frequencies. Smooth lines are model (D) fits for the 2 measurements as follows: C = 80 pF, $R_e = 526 \, \text{M}\Omega$, $C_e = 13.5 \, \text{M}\Omega$, $C_{se} = 3.5 \, \text{pF}$, and $C_{se} = 5.6 \, \text{pF}$ for the low and high solution levels (bottom and top curves), respectively. D: schematic diagram of electronic and electrode model.
frequency range. The effect of the electrode on the frequency domain functions is shown in Fig. 7, B and D (see following text). As mentioned in the following, the passive electrotonic parameters were determined with one-half the frequency range and fixed electrode parameters. The electrode parameter values during the whole cell recording are given in Table 1. The average increase of \( R_e \) from 10 to 17 M\( \Omega \) is likely due to plugging of the electrode by membrane fragments and cytoplasmic material during the breakthrough of the membrane. The decrease in the electrode capacitance may be related partially to different factors, namely, the attachment of membrane fragments on the inner cell wall at the tip, variations in solution levels, or a need for a distributed capacitance to accurately model the electrode. Because parameter estimation done with more complicated electrode models (Major et al. 1992) did not significantly alter the electrotonic parameters, all of our analyses were done with an electrode modeled as a single resistance, \( R_e \), and a nondistributed capacitance, \( C_e \) (see APPENDIX). It is worth noting that the determination of the electrode capacitance is a critical part of an accurate estimation of the soma capacitance. In this regard, electronic compensation methods are especially subject to error because overcompensation can reduce part of the observed dendritic capacitance, which is seen from the soma through a series resistance like the electrode itself.

Thus once the electrode properties are known, it is possible to separate the properties of the attached electrode from those of the neuron and evaluate the electrotonic structure. This itself requires a valid theoretical formalism for the dendritic tree to obtain the excitability properties of both the soma and dendrites.

### Data analysis and rationale

The goal of these experiments is to analyze dendritic membrane excitability. The rationale of the analysis is to use different types of measurements that allow the determination of the physiological electrotonic structure, both active and passive. The basic steps in the analysis consist of the following: 1) determination of passive electrotonic structure at hyperpolarized membrane potentials with a linear analytic model, i.e., having perfect spatial resolution. 2) Evaluation of compartmental models at different membrane potentials to evaluate the required number of compartments needed to achieve adequate spatial resolution during the activation of ionic conductances. 3) The analysis of real-time kinetic responses with compartmental models that are constrained by the passive electrotonic structure.

**THEORETICAL CONSIDERATIONS—ANALYTIC VERSUS COMPARTMENTAL MODELS.**

**Linear-analytical models—resting neurons.** The experimental protocol described in the preceding text involves an analysis of both large step nonlinear data and small-signal linearized responses. Our goal is to obtain a minimal model that accurately describes the soma and dendritic behavior observed in the measured neurons. It was found that the dendritic tree could be well described by a single Rall equivalent cylinder, thus avoiding the need to use more complex models based on detailed morphology. Models with two dendritic cables connected to the soma did not significantly improve the error of the fit. This result supports the hypothesis that the branching dendritic structure of *Xenopus* neurons follows the impedance matching criteria at branch points developed by Rall (1960). More complex morphological models are only needed when the branch point matching criteria are not met. Although anatomic measurements on fixed tissue can indicate such a discrepancy, it is not clear if these estimates always apply to living tissue. Electrophysiological measurements, in both the time and frequency domains from neurons in their normal physiological state, can provide data to evaluate the accuracy of collapsed dendritic models and as such are likely to be more suitable for determining the adequacy of simplified dendritic models to describe data than anatomic measurements from fixed tissues. The ability of a single uncoupled model to adequately describe our data also demonstrates that the neurons are not likely to be electrotonically coupled (Perrins and Roberts 1995a). Thus because interneurons do not show electrotonic coupling (Perrins and Roberts 1995b), our analysis further supports the presumption that the selected cells are from this class of neurons.

The implementation of the collapsed dendritic cylindrical model has been done with analytic (Major 1993a,b) and compartmental models (Rapp et al. 1994). However, linear analytic models (see APPENDIX) are advantageous because they have perfect spatial resolution and are computationally efficient. The applicability of these models in different neurons was determined by comparing the analytic and compartmental formulations as a function of number of compartments. Near the resting potential there was good agreement using from three to five compartments.

The passive electrotonic structure always was determined near the resting potential with both passive and active linearized responses (Borst and Haag 1996; Major et al. 1994; Surkis et al. 1998). Under these conditions, an analytic model equivalent to an infinite number of compartments was used (see APPENDIX). Although the linear analytic model avoids errors due to spatial resolution, it is only valid near the resting potential or under experimental conditions such that the neuron is entirely passive. It should be emphasized that linearized models are not necessarily passive and are capable of describing the kinetic behavior of the voltage-dependent conductances (Moore and Buchanan 1993) over a limited potential range if the steady-state potentials throughout the cable are relatively constant (Murphey et al. 1995).

**LINEAR COMPARTMENTAL AND ANALYTIC MODELS—DEPOLARIZED NEURONS IN STEADY STATE.** At depolarized potentials the steady-state potential profile inherent in the dendritic structure leads to variable activation of the ionic conductances. This requires a compartmental model to correctly determine voltage-dependent admittance functions at each dendritic location (see APPENDIX). Nevertheless the analytic model is a good approximation at moderate depolarizations and can provide an excellent initial estimate of the final impedance. This point is of computational importance because calculation of the analytic impedance can be orders of magnitude faster than compartmental estimates.

The number of compartments necessary to assure adequate spatial resolution is clearly a function of both the electrotonic structure and the nature of the ionic conductances (Bush and Sejnowski 1993). At rest or with a passive neuron, the best test of the compartmental model is a comparison with the analytic solution. However, active neurons at depolarizing potentials require increasing the number of compartments until no significant change occurs. Under these depolarized conditions, the compartmental model then can be used to evaluate the adequacy of the analytic model. For many neurons, the electrotonic structure was sufficiently compact that very little error occurred with the analytic model at depolarized potentials. Significantly larger errors occurred with compartmental models having too few segments than ever observed due to a steady-state potential profile error.

**NONLINEAR COMPARTMENTAL MODELS—LARGE SIGNAL KINETIC ANALYSIS OF IONIC CURRENTS.** The analysis of the constant current responses clearly requires nonlinear kinetic equations; however, this is also true for a somatic voltage clamp because of the effects of the dendritic membrane potential transients. These kinetic models have
been described extensively by numerous authors (Koch and Segev 1998), and our specific implementation is given in the APPENDIX.

Our initial analysis was done with the minimum number of three dendritic compartments in which the passive electrotonic parameters (ratio of dendritic to somatic surface areas, \( A \), and electrotonic length, \( L \)) were identical to those of the analytic model (see APPENDIX). The spatial resolution of the compartmental model then was evaluated by increasing the number of compartments until minimal differences were obtained. In general, the resting neuron was described adequately by a three-compartmental dendritic model; however, \( \geq 10 \) compartments were essential to describe voltage-clamp currents at depolarized potentials when the ionic conductances are significantly activated (Bush and Sejnowski 1993; D’Aguano et al. 1989). A dendritic model with 30 compartments was usually indistinguishable from the analytic model.

In brief, the parameter estimation methods, as previously described (Murphey et al. 1995, 1996), were used in the following order: The passive electrotonic and electrode parameters initially were estimated at resting or hyperpolarized potentials with a linear analytic model (see APPENDIX) over the entire frequency range. Parameter estimation was done by an iterative gradient descent method. Fits in a particular minimum were considered adequate if the minimal error change was \(<0.1\%\). In addition local minima were avoided by the use of both the frequency and time domain data under voltage and current clamp. Afterward, the electrode parameters were fixed and the electrotonic parameters were estimated over one-half the frequency range. The validity of a three-compartmental dendritic model also was confirmed for the passive membrane by demonstrating an adequate fit of the small signal real-time data. The data then were fitted simultaneously in the frequency and time domains with the linear analytic model and a three-dendritic compartmental model, respectively. The adequacy of both the frequency and time domain fits were compared directly with a 30-compartmental model. If the fitting criteria were not met, the data were fitted again with the higher resolution compartmental model that was implemented using the FindMinimum and FindRoot procedures of Mathematica (Wolfram Research, Champaign, IL). These procedures then were applied to the active conductances after fixing the passive parameters. Multiple records at different membrane potentials were analyzed simultaneously in both the time and frequency domains. If necessary, the electrode parameters, \( R_e \) and \( C_e \), were refitted during the course of the experiment; however, the electrotonic parameters remained at their original estimated values. In this case, constraining limits based on measured electrode parameters (Fig. 4) were placed on \( C_e \) and \( R_e \), as follows: \( C_e (2–6 \text{ pF}) \) and a minimum for \( R_e \) of 6–10 MΩ, which was measured in the bath before the patch was made.

In summary, the approach developed in this paper is to analyze both whole cell voltage- and current-clamp data with a real-time three-dendritic compartment nonlinear model that is constrained simultaneously by the linear analytic frequency domain form of the same model. These two forms of the neuronal model are computationally efficient because of the small number of compartments in the former and the analytic representation of the latter. The number of compartments then is increased to evaluate the errors due to inadequate spatial resolution for the real-time transients and the dendritic potential profile in the steady state.

**RESULTS**

**Whole cell action potentials**

All neurons perfused with the potassium electrode solution had resting potentials more negative than \(-55 \text{ mV}\) and action potential magnitudes that reached overshooting positive values. The action potential behavior varied between a Type I repetitive firing behavior to a Type II single action potential in response to a maintained constant current. Figure 5A shows a single action potential from a Type I neuron responding to a just threshold stimulus. Increasing the stimulus strength increased the number of action potentials (Fig. 5B) and the average firing rate. In general, the cells responded repetitively with minimal accommodation to a maintained constant current that was twofold or greater than threshold (Fig. 5C). Figure 5D illustrates superimposed constant current steady-state current voltage (I-V) curves for nine neurons showing this type of action potential behavior. A marked rectification is observed where the maximal slope conductance is reached at about \(-40 \text{ mV}\).

About 1/4 of the neurons, referred to as Type II neurons, showed marked accommodation in their firing responses. Figure 6, A–C, illustrates two examples of this type of behavior. The most extreme form is seen in Fig. 6A in which a single action potential is evoked by constant current stimulation \( \pm 10 \) times threshold. An alternative type of adaptation is seen in Fig. 6, B and C, in which a constant current evokes a series of action potentials that decrease in size and finally cease. These neurons show increased frequencies and numbers of action potentials as the current increases (Fig. 6C); however, the responses are not maintained. The constant current voltage curves of Type II neurons show a significant rectification over a wide potential range (Fig. 6D) leading to a maximum slope conductance at a more positive potential than seen in Type I neurons.

**Passive electrotonic or structural parameters**

The analysis of electrotonic behavior provides information about the passive structure of a neuron on which active prop-
In addition to problems associated with properties of the dendritic tree is notoriously difficult (Spruston and Johnston 1992). In the intact cell to ascertain the actual electrotone behavior. Ideally morphology could provide the basis of the model used for interpreting electrophysiological measurements; however, such models can require large numbers of compartments and are computationally cumbersome. Our approach is to obtain a minimal model that is consistent with the morphology and rigorously satisfies the spatial resolution requirements imposed by the electrophysiologically measured cable properties.

To measure passive neuronal properties, data at hyperpolarized membrane potentials were obtained in the presence of TTX during internal perfusion of potassium gluconate or cesium sulfate. The electrode properties were analyzed in conjunction with those of essentially passive neurons in a sequential manner such that fitted electrode parameters were fixed before finally evaluating the passive electrotonic structure (see METHODS). Neurons perfused with cesium sulfate had unstable resting potentials and survived better if voltage clamped at −60 mV as rapidly as possible after establishing the whole cell recording. Removal of the potassium conductance was presumably responsible for the instability of the resting potential and often led to maintained depolarized potentials that damaged the neurons. The d.c. impedance of these neurons often exceeded 10 GΩ.

Figure 7A illustrates the potential time course in response to a hyperpolarizing current for a virtually passive cesium perfused neuron. A multiexponential analysis of this response to obtain the membrane time constant and associated electrotonic properties of the dendritic tree is notoriously difficult (Spruston and Johnston 1992). In addition to problems associated with separating exponential functions, the properties of the electrode are difficult to assess. The corresponding measurements done in the frequency domain (Fig. 7B) provide more sensitivity than real-time measurements for the estimation of electrode and electrotone parameters. The fits shown in Fig. 7 indicate good agreement for both the real-time and frequency domain current-clamp data. Figure 7, C and D, shows voltage-clamp data and model fits for the same neuron using the model parameters that describe the current-clamp data (Fig. 7, A and B) with the notable exception of $g_{\text{soma}}$, which was reduced to one-half its value. This difference is likely due to the incomplete exchange of cesium and potassium ions during the constant current measurement that was done at the beginning of the experiment. Otherwise the parameters are identical and show that the two measurements are equivalent as is demonstrated in subsequent figures from other neurons. The effect of the electrode on the impedance functions also is illustrated for both the current- (Fig. 7B at −70 mV) and voltage-clamp (Fig. 7D at −63 mV) frequency domain experiments. The only significant changes were in the phase functions showing a deviation in the phase at high frequencies when the electrode was removed.

The mean values (Table 2) of the passive electrotone parameters of cesium perfused neurons show a soma capacitance of ~3 pF and a dendritic structure that was approximated by a single equivalent cylinder having an electrotonic length of 0.25 and a dendritic to soma area ratio of 3.6. Assuming that the neuron is a perfect sphere with a specific capacitance of 0.5–1 μF/cm², then its diameter would be 10–14 μm, respectively. These estimates are consistent with visual observations of these neurons during the experiments (see Fig. 2). The majority of experiments were done at stage 47; however, some measurements at stages 42–46 showed that the electrotonic parameters between these stages are not radically different (Table 4). The results of this study should apply to all stages between 42 and 47; however, it is clear that dendritic structures are in different states of development during these various stages.

Voltage-gated potassium conductances

Because part of the resting conductance is due to the potassium conductance, it is often difficult to determine the passive electrotonic structure with real-time hyperpolarizing pulses (Spruston and Johnston 1992; Surkis et al. 1998). Thus the effects of voltage-dependent conductances must be generally taken into account in resting neurons. We have been able to demonstrate that hyperpolarized interneurons perfused with cesium or potassium ions are essentially passive; however, at the resting potential most voltage-dependent conductances can contribute to the linear properties. Therefore passive electrotonic parameters of a normal resting neuron must be estimated in the presence of active conductances. Thus it is necessary to assess the distribution of ionic channels between the soma and dendritic tree. Although morphological labeling methods can indicate receptor distributions, it is important to functionally estimate these distributions. This is not generally possible by direct measurements in intact tissues; however, in our analysis, a homogenous distribution of ionic channels was shown to be adequate for the experimental conditions explored. This result is of some significance because it has been demonstrated previously that the frequency domain method is capable of...
detecting differences in the spatial distributions of activated receptors (Murphey et al. 1995); however, extremely accurate frequency domain measurements with averaging would be required before spatial effects can be seen easily. This was not done in these experiments.

Figure 8 illustrates data obtained in the presence of TTX for both current- and voltage-clamp modes and provides a stringent test of the full nonlinear model containing a single voltage-dependent potassium conductance throughout the dendritic tree. The time course of the hyperpolarizing potential response during K-glutamate perfusion is more rapid than observed in the corresponding cesium perfused neuron of Fig. 7. These results support the hypothesis that part of the conductance at rest is due to the voltage-dependent potassium conductance. The depolarizing potential response was lower in magnitude and also faster due to the increased activation of the potassium conductance (Table 3). Thus the normal resting conductance has both voltage-dependent and -independent (leakage) components (Surkis et al. 1998).

The corresponding frequency domain data and fitted curves (Fig. 8, B and D) show that the model parameters provide a good description of both the passive and active parameters. As with the passive case of Fig. 7, the voltage-clamp responses (Fig. 8, C and D) are shown to be well predicted by the current-clamp model of Fig. 8, A and B. These results confirm that the voltage clamp is adequately controlling the soma potential and provides verification that the dendritic model used for both the current- and voltage-clamp experiments is an accurate description. The electrotonic length of this neuron was 0.25 and was sufficiently small to allow the three-compartment dendritic model to accurately describe the voltage-clamp currents. The compactness of the dendritic tree also allowed observation of putative excitatory inward synaptic currents that decrease in amplitude during depolarizing voltage-clamp steps.

The steady-state electrotonic structure of the dendrite is given by a minimum of three parameters, the soma conductance ($g_{\text{soma}}$), electrotonic length ($L$), and the ratio of the dendritic to somatic areas ($A$). Differences among neurons that might occur due to the dendritic structure should be revealed by a grouping of these neurons according to these variables (see Table 4). The three dimensional plot of these variables in Fig. 9 shows that there is a clustering of neurons (stars) toward the front corner of the $A$ versus $L$ surface defined by $A > 4$ and
have a higher average broader range of all three parameters; however, no neurons were measured. Because the correlation of electrotonic structure neurons represent neurons have a larger dendritic to soma area ratio of 6.5; however, with normal internal potassium ions, 1.70 3.32 0.05 0.17 0.71 3.09 0.89 5.29 13.44 3036 ± 1388 0.12 0.31 0.05 0.21 3.05 0.80 6.50 15.47 245 1.67 3.34 0.12 0.36 0.05 0.30 0.17 9.63 9.27 5.18 6.61 Mean n = 12 8 20 C_soma, pF 3.24 ± 1.47 2.87 ± 0.71 3.09 ± 1.21 E_length 0.35 ± 0.15 0.25 ± 0.12 0.31 ± 0.14 Aratio 4.94 ± 2.36 3.60 ± 1.74 4.40 ± 2.19 g_soma, PS 167 ± 266 83.2 ± 50.2 134 ± 209 E_leak, mV −58.00 ± 4.95 −54.93 ± 15.23 −56.77 ± 10.10 R_sea, MΩ 2029 ± 1319 3187 ± 1437 2493 ± 1451

Values are means ± SD. L < 0.3. This Type B group (stars) has a high-input impedance and a g soma < 50 pS. The remaining Type A (circles) has a broader range of all three parameters; however, no neurons were found that had A > 4 when L > 0.4. The Type A neurons have a higher average g soma and electrotonic length in potassium gluconate compared with cesium sulfate perfused cells (Table 2 and encircled symbols in Fig. 9). This difference is consistent with the assumption that removal of potassium ions decreases the passive dendritic conductance and correspondingly decreases the electrotonic length. Thus part of the variation observed in the Type A neurons is probably due to the cesium perfusion, which suggests the average value of L with normal internal potassium ions is more physiological. Type B neurons have average g soma and electrotonic lengths that are both lower than Type A neurons and independent of the internal potassium concentration (Table 2).

The excitability properties of these two classes, Type A and Type B, appear to be correlated with the Type I and II classification of Figs. 5 and 6. The closed symbols in Fig. 9 indicate neurons in which the correlation was confirmed. The firing properties of the remaining neurons were not measured. The means of these two types are given in Table 2 show that the Type A neurons have an average electrotonic length of 0.36 and a dendritic to soma area ratio of 3.3; however, with normal internal potassium ions, L = 0.44 and A = 3.64. The Type B neurons have a larger dendritic to soma area ratio of 6.5; however, a smaller electrotonic length of 0.21. These latter neurons represent ~30% of the presumed interneurons that were measured. Because the correlation of electrotonic structure with excitability properties required consideration of both L and A, it would be unlikely that only the morphological area of the dendritic tree would distinguish neurons with different firing properties.

### Spatial resolution and dendritic potential profile

The potassium conductance near rest is well described by a three-compartmental dendritic model in real time and the analytic model for the frequency domain; however, major discrepancies can occur at large depolarizations. As discussed in METHODS, as the effective electrotonic length increases because of the activation of voltage-dependent conductances, the number of compartments must be increased until there are no changes in the linear and nonlinear responses of the model equations. Under these conditions, the compartmental models can be used to obtain an increased spatial resolution for the transient response and the steady-state dendritic potential profile for the frequency domain. Figure 10A illustrates a neuron having a passive electrotonic length of 0.5 where the time domain fits for a three-dendritic compartment model (—) are progressively worse with depolarization. At these potentials, the effective electrotonic length is considerably >0.5, which means that the level of depolarization of the peripheral dendrites is considerably less than the soma. Therefore the spatial resolution of three-compartmental model for this data are not adequate and cannot quantitatively describe the voltage-clamp results. Curiously, the predictions of a three-compartmental model show less current than measured, probably because the lack of spatial resolution leads to an abnormally low level of depolarization in the end compartments that produces less current than observed with more compartments.

Figure 10A, - - -, shows a marked improvement of the model, using the same passive and active parameters, when the number of compartments for the real-time response was increased to 30. The frequency domain fits (Fig. 10B) show that the analytic (—) and 30 compartmental (- - -) models show better agreement with the data near the resonant peak; however, the low-frequency impedance of the analytic model matches the data better. The decreased magnitude at low frequencies of the analytic model compared with the compartmental model is not due to spatial resolution errors but occurs because the analytic model assumes a uniform potential throughout the neuron. The increased low-frequency impedance magnitude of the 30-compartmental model is a consequence of the reduced activation of the potassium conductance in the peripheral compartments as would be expected because of the increased electrotonic length. The analytic and 30-compartment models superimpose at the resting potential; however, a frequency domain model with only three dendritic compartments is completely inadequate at all membrane potentials. The relatively good agreement between the analytic and 30-compartmental model frequency domain fits suggests that spatial resolution errors are more significant than those due to the potential profile. Although we used 30 compartments, 10 compartments were generally sufficient for most neuronal structures (Bush and Sejnowski 1993; D’Agano et al. 1989). Nevertheless, we emphasize that the preceding procedure is a relatively simple way to evaluate the correct number of compartments for a particular set of conductances and consequently is preferred to assuming a fixed number.

The neuron of Fig. 10 also shows a marked linear impedance resonance that is determined by the interaction of the relaxation time of the potassium conductance and the passive electrotonic properties. The half-activation potassium conductance time
constant, $t_n$, is of the order of milliseconds, which leads to transients that cannot be resolved in the real-time measurements; however, because a 1-ms time constant has the corner frequency of 160 Hz, it is possible to estimate this parameter from the impedance data. Thus the activation time constant principally was determined by the frequency domain resonance, which is sensitive to its value. Both Type A and B models do show impedance resonances; however, the Type B model shows a resonance at more depolarized potentials. This simulation result is consistent with our observation that, over a limited range of membrane depolarizations, resonance was more frequently observed in Type A than B neurons.

The slow decay seen in the voltage-clamp currents was not modeled; however, we have obtained essentially identical activation time constants with an inactivating potassium conductance model. An additional cause of the slow decay in the current could be a change in the internal concentration of potassium ions because these neurons are relatively small compared with other preparations. In general, the potassium conductance has a positive slope conductance; however, an inactivating potassium conductance could in principle show a negative slope conductance. Tables 3 and 5 show the results for the fast voltage-dependent potassium conductance for the two groups of neurons, Type A and Type B.

**DISCUSSION**

The passive membrane properties of the larval Type A and B neurons showed input resistances ($R_m$) of 1–3 GΩ that was measured at −70 mV (Table 2). These values are slightly higher than the measured resistances of embryonic neurons,
TABLE 4. Electrotonic passive parameters for individual neurons

<table>
<thead>
<tr>
<th>Neurons*</th>
<th>Solution</th>
<th>(r_c), M(\Omega)</th>
<th>(c_e), pF</th>
<th>(C_{\text{soma}}), pF</th>
<th>(E_{\text{length}})</th>
<th>(A_{\text{ratio}})</th>
<th>(g_{\text{soma}}), pS</th>
<th>(V_{\text{leak}}), mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Passive parameters of Type A cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>47</td>
<td>97J01A</td>
<td>K⁺</td>
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<td>3.95</td>
<td>0.479</td>
<td>2.89</td>
<td>152</td>
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<td>97J31J</td>
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<td>−58</td>
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<td>1.77</td>
<td>135</td>
<td>−59.81</td>
</tr>
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<td>987</td>
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</tr>
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<td>2.18</td>
<td>0.492</td>
<td>3.62</td>
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<td>2.04</td>
<td>0.447</td>
<td>3.27</td>
<td>57.8</td>
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<td>B. Passive parameters of Type A cells with a Cs⁺ intracellular solution</td>
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<td>2.53</td>
<td>59.2</td>
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</tr>
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<td>C. Passive parameters of Type B cells</td>
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<td>0.193</td>
<td>5.74</td>
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<td>2.00</td>
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<td>0.234</td>
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<td>7.224</td>
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<td>Cs⁺</td>
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<td>2.39</td>
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<td>43</td>
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<td>47.8</td>
<td>3.76</td>
<td>2.36</td>
<td>0.207</td>
<td>4.89</td>
<td>60.1</td>
</tr>
</tbody>
</table>

* Numbers (i.e., 47, 46, 45, etc.) represent stages of development.

which were generally 1 GΩ (Dale 1991). Embryonic soma are somewhat larger than those of the larva; however, the dendritic structure would tend to reduce the effect on the input resistance of the smaller soma. Estimates of the embryo soma capacitance vary from 10 to 120 pF (Dale 1995b; Prime et al. 1998; Roberts and Tunstall 1990; Soffe 1990), possibly reflecting a large distribution of sizes due to different developmental states and measurement conditions. A value of 9.5 pF was used by Dale (1995b) in model simulations that were based on measurements of isolated embryonic neurons. Furthermore isolated larval neurons have been reported to have a lower cell capacitance of 3.2 pF (Sun and Dale 1998). This value is quite comparable to our soma capacitances of 3.3 and 2.6 pF for Type A and B neurons, respectively (Table 2). In our measurements of intact neurons, there is a significant additional dendritic capacitance that was 4–7 times that of the soma. Thus the total capacitance of the intact larval neuron is considerably larger than the isolated neuron.

The choice of a correct dendritic model is dependent on the electrotonic structure and how it might change dynamically. If the assumption of a single equivalent cylinder is reasonable, then the analytic model provides the best linear description, which can be passive or active. We have shown that the resting neurons in the Xenopus larva can be described by an analytic model and that a three-dendritic compartmental model is usually adequate for real-time response. This small number of compartments is not adequate for depolarized neurons because the dynamic electrotonic length increases and the voltage-clamp currents cannot be described correctly. We have found that 10–30 compartments is sufficient for describing the real-time behavior of voltage-clamped Xenopus larval neurons.

The relative contribution of the dendritic cable to the small-signal passive conductance often is referred to as \(\rho\), namely, \(g_{\text{dendrite}}/g_{\text{soma}}\) where \(\rho = (AIL) \tanh L\) in our terminology (APPENDIX). For the range of \(L\) values found in these experiments, \(\rho\) is nearly equal to \(A\). We were not able to find correlations between active properties and individual electrotonic parameters, such as \(A\) or \(L\); however, the two together do seem to allow the separation of a particular group, Type B, from the remaining Type A neurons. The mean parameter values of these groups suggest that the larger dendritic area of the Type B neuron is associated with a lower electrotonic length compared with Type A neurons. This relationship enhances inte-
egrative mechanisms because the larger dendritic region is elect

trotonically closer to the soma. Even Type A neurons do not
appear to have electrotonic lengths >0.4 for dendritic area
ratios >4.

The membrane conductance of neurons at rest appears to be

different for Type A and B neurons. In contrast to Type B
neurons, Type A neurons have a component of the resting
passive conductance that is dependent on potassium ions in
addition to the voltage-dependent potassium conductance. Our
electrotonic analysis separates active and passive properties
and should show a different value of \( L \) for neurons having
similar dendritic areas and different passive \( g_{soma} \)'s. Table 2
indicates that \( L \) is lower for Type A neurons when \( g_{soma} \) is
reduced by cesium compared with control values with normal
internal potassium ions. Furthermore Fig. 9 shows that \( g_{soma} \)

increases with \( L \) and decreases with \( A \), as is suggested by the
relationships between \( g_{soma} \), \( A \) and \( L \) given in the APPENDIX.
Correspondingly, Type B neurons do not show a change in
\( g_{soma} \) or \( L \). The demonstration that Type A neurons show a
lower \( L \) in the presence of cesium ions supports the hypothesis
that the membrane resistivity of the dendritic membrane is
similar to that of the soma. If the cesium perfused neurons were
removed from Fig. 9, the Type A neurons would be more
homogenous in their properties and would show a greater
difference in electrotonic length compared with Type B neu-
rons.

In the Type B group, action potentials showed marked
adaptation and occasionally remained depolarized with small
levels of injected current. The action potential behav-
ior was measured in 8 of the 19 neurons of Fig. 9, as

\[
\text{FIG. 9. Distribution of neuronal types based on}
\text{electrotonic structure. Coordinates of the 3-dimen-
sional plot were the d.c. electrotonic parameters:}
\text{soma conductance (} g_{soma} \text{), electrotonic length (} L \text{),}
\text{and the ratio of the dendritic to soma areas (} A \text{).}
\text{Clustering of the star symbols provides a way to}
\text{define Type A (circles) and Type B (stars) neurons.}
\text{All neurons in which the firing behavior was mea-
sured are indicated by filled (8 of 19) symbols. One}
\text{Type I, A neuron was not shown because one of its}
\text{coordinates (} g_{soma} \text{), is out of the range (Table 4,}
\text{97KO5C) of the graph. In each instance, there is a}
\text{strict correlation between Type A and Type I mul-
tiple firing neurons or Type B and Type II accommodating}
\text{neurons. Circled symbols represent neu-
rons perfused with cesium sulfate, which in the case}
\text{of Type A neurons usually show a lower value of}
\text{L.}
\]

\[
\text{FIG. 10. Influence of electrotonic structure on potas-
}\text{sium currents. A: voltage-clamp currents at 3 levels of depolar-
ization. B: corresponding impedance plots for the each volt-
age-clamp step fitted with an analytic model. A significant}
\text{discrepancy is seen between the fit of the 3-compartmental}
\text{model in A, suggesting a need for increased spatial resolu-
tion. Dashed lines show an improved model fit for the}
\text{increase in number of compartments from 3 to 30. Other}
\text{parameters of the model were identical to those used in the}
\text{3-compartmental model. Parameters were } C_{soma} = 3.95 \text{ pF;}
\text{ } L = 0.479; \text{ } A = 2.89; \text{ } g_{soma} = 0.15 \text{ nS; and } V_{peak} = -57.5
\text{ mV. Electrode model parameters are } C_e = 2.9 \text{ pF and } R_e =
\text{ 25 MΩ. Active parameters were } g_K = 4.34 \text{ nS; } v_K = -90
\text{ mV; } v_n = -30.2 \text{ mV; } s_n = 0.031 \text{ mV }^{-1}; \text{ } t_n = 2.4 \text{ ms; } r_n =
\text{ 0.02 mV }^{-1}. \text{ Neuron 97J01A, Type A.}
\]
indicated by the filled symbols. In each instance, there was a match between Types A and B and Types I and II, respectively (Figs. 5 and 6). The Type II accommodating neurons also have been observed at stage 37/38 (Roberts and Sillar 1990) for dorsolateral commissural (dlc) neurons in contrast to single impulse responses from ventral interneurons. Because this behavior appears to be correlated with dendritic structure in our measurements of larvae, it is tempting to speculate that embryonic dlc interneurons may have a significant dendritic structure.

The differences between Type I and II neurons also are reflected clearly in the I-V curves. Similar behavior for central cochlear neurons has been observed in brain slices of the guinea pig cochlear nucleus (White et al. 1994). Both the high passive resistance and steeper potassium conductance activation curve give Type B neurons more steady-state rectification. The lower \( g_{\text{soma}} \) would allow steady-state negative conductances due to sodium and calcium ions to influence the I-V curve, especially for the condition that the potassium current is not activated strongly at moderate depolarizations. Although Type A and B neurons have similar half activation potentials, the steeper slope of the Type B activation curve delays activation of the potassium conductance. This would be manifested in an I-V curve by an abrupt increase in current near the half activation potential, as is observed in the I-V plots for Type II neurons.

In summary, the analysis of these experiments has provided a quantitative description of the passive electrophysiological properties of putative spinal interneurons of Xenopus larvae that consist of a soma with one equivalent dendritic cable. The models with a limited number of compartments are remarkably accurate for the different types of neurons; however, active conductances are likely to require increasing numbers of compartments because of dynamical variations in the space constant. The use of an analytic model provides a more systematic and accurate way to determine the number of compartments needed for describing the active properties of any given neuron.

**APPENDIX**

**Model equations**

As described previously (Borg-Graham 1991; Moore and Buchanan 1993; Moore et al. 1999; Murphey et al. 1995), the kinetic formulation using \( x \) as a generalized kinetic variable is

\[
I_i = I_s + I_{\text{exc}} + I_{\text{inj}} \quad (A1)
\]

\[
I_s = g_{\text{soma}}(V_i - V_s) \quad (A2)
\]

\[
I_{\text{exc}} = \sum_p g_p(V_i - V_p) \quad (A3)
\]

\[
I_{\text{inj}} = g_{\text{core}}(V_i - V_{i+1}) \quad (A4)
\]

\[
\frac{\partial V_i}{\partial t} = -\frac{N}{A_{\text{soma}}} I_i \quad (A5)
\]

\[
\frac{\partial x}{\partial t} = \alpha_x(1 - x) - \beta_x = (x_a - x)/\tau_x \quad (A6)
\]

\[
\tau_x = 1/(\alpha_x + \beta_x) \quad (A7)
\]

\[
x_a = \alpha_x/(\alpha_x + \beta_x) \quad (A8)
\]

\[
\alpha_x = (1/\tau_x) \exp(V - v_i)(2x_i - r_i) \quad (A9)
\]

\[
\beta_x = (1/\tau_x) \exp(V - v_i)/(2x_i + r_i) \quad (A10)
\]

where \( c_{\text{soma}} \) is the capacitance of the soma, \( I_s \) is the current in the \( i \)th compartment, \( I_{\text{exc}} \) is the current between compartments, \( V_s \) is the membrane potential in the \( i \)th compartment, \( g_{\text{soma}} \) and \( I_I \) represent a nonspecific leakage conductance and current having \( V_s \) as a reversal potential, \( A \) is the total area of the dendritic compartments to the soma, \( N \) is the number of compartments, \( g_p \) is a generic voltage-dependent ionic conductance with a reversal potential of \( V_p \), and whose kinetics is governed by the unitless variable, \( x \), which has a steady-state value of \( x_a \). Thus at the half-activation \((x_a = 1/2) \), voltage, \( v_i \) and \( s_i \) is the slope of \( x_a \), \( \tau_s \) is the time constant, \( \tau_x \) and \( r_i \) is the normalized slope of \( x \). In this paper \( g_p \) represents potassium \((g_K) \) where the variable, \( x \) is \( n \).

The analytic linearized admittance for one variable is given as

\[
Y_{\text{soma}}(V,f) = j2\pi f c_{\text{soma}} + g_{\text{soma}} + \sum_p g_p(x_s(V) + (V - V_p)\delta x(V,f)) \quad (A11)
\]

\[
\delta x(V,f) = \frac{\partial x}{\partial V} x_s - \left( \frac{\partial x}{\partial V} x_s - \frac{\partial x}{\partial V} x_s \right) \right) / (2j\pi \tau_s + 1) \quad (A12)
\]

or

\[
Y_{\text{soma}}(V,f) = j2\pi f c_{\text{soma}} + g_{\text{soma}} + \sum_p g_p(x_s(V) + (V - V_p)(dx_s/dV))/(1 + j2\pi \tau_s) \quad (A13)
\]

where \( j = \sqrt{-1} \) and \( f = \) frequency in Hertz. The compartmental model was recursively computed from the end compartment, either in a simple loop or symbolic notation (see Murphey et al. 1995) using Mathematica (Wolfram Research, Champaign, IL). Alternatively, an
analytic model was used (see Moore et al. 1999; Rall 1960), which leads to an active cable with an admittance, \( Y_a \), as follows

\[
Y_a = Y_{\text{soma}} + \frac{A g_{\text{soma}} Y_{\text{dendrite}}}{L} \tanh \left( \frac{L Y_{\text{soma}}}{g_{\text{dendrite}}} \right)
\]

where \( \lambda \) is the space constant, \( L \) is the electrotonic length, \( \lambda/L \), \( N \) is the number of compartments and \( \lambda = NL \), is in units of \( N \). Note that the units of the hyperbolic tangent term are given by \( g_{\text{dendrite}} \). In a completely passive neuron at d.c., the dendritic conductance, \( g_{\text{dendrite}} = (A g_{\text{soma}}/L)^2 \tanh L \). Finally, the total admittance with the electrode properties is

\[
Y = 2\pi f C_e + Y_f (1 + R_e Y_e)
\]

where \( C_e \) is the electrode capacitance and \( R_e \) is the electrode resistance.

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