Active Dendritic Membrane Properties of *Xenopus* Larval Spinal Neurons Analyzed With a Whole Cell Soma Voltage Clamp

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Saint Mieux, Benoit and L. E. Moore. Active dendritic membrane properties of *Xenopus* larval spinal neurons analyzed with a whole cell soma voltage clamp. *J. Neurophysiol.* 83: 1381–1393, 2000. Voltage- and current-clamp measurements of inwardly directed currents were made from the somatic regions of *Xenopus laevis* spinal neurons. Current-voltage (*I*-*V*) curves determined under voltage clamp, but not current clamp, were able to indicate a negative slope conductance in neurons that showed strong accommodating action potential responses to a constant current stimulation. Voltage-clamp *I*-*V* curves from repetitive firing neurons did not have a net negative slope conductance and had identical *I*-*V* plots under current clamp. Frequency domain responses indicate negative slope conductances with different properties with or without tetrodotoxin, suggesting that both sodium and calcium currents are present in these spinal neurons. The currents obtained from a voltage clamp of the somatic region were analyzed in terms of spatially controlled soma membrane currents and additional currents from dendritic potential responses. Linearized frequency domain analysis in combination with both voltage- and current-clamp responses over a range of membrane potentials was essential for an accurate determination of consistent neuronal model behavior. In essence, the data obtained at resting or hyperpolarized membrane potentials in the frequency domain were used to determine the electrotonic structure, while both the frequency and time domain data at depolarized potentials were required to characterize the voltage-dependent channels. Finally, the dendritic and somatic membrane properties were used to reconstruct the action potential behavior and quantitatively predict the dependence of neuronal firing properties on electrotonic structure. The reconstructed action potentials reproduced the behavior of two broad distributions of interneurons characterized by their degree of accommodation. These studies suggest that in addition to the ionic conductances, electrotonic structure is correlated with the action potential behavior of larval neurons.

**INTRODUCTION**

Knowing the active properties of *Xenopus* spinal neurons is essential for an understanding of the role of voltage-dependent ionic conductances on action potential behavior. Whole cell patch-clamp measurements of isolated neurons from the embryo have been used to construct a single-compartment neuronal model (Dale 1995a) that was used in neural network simulations to provide a realistic description of locomotor behavior (Dale 1995b). These simulations demonstrate the importance of quantitative measurements for a better understanding of emergent system behavior and suggest that a single somatic compartment may be a reasonably sufficient model for the *Xenopus* embryo at stage 37/38 (Dale and Kuenzi 1997; Prime et al. 1998; Soffe 1990; Wolf et al. 1998). Because *Xenopus* larvae neurons have extensive dendritic trees (Van Mier et al. 1985), it is necessary to develop a different analysis to quantitatively describe their active properties, especially those of the dendritic membrane. Although measurements from small patches or fragments of dendritic membranes (Kavalali et al. 1997) provide useful data on individual channels, it is difficult to determine the overall effect of membrane conductances on the whole cell behavior from just the kinetic description of a patch of membrane (see Destexhe et al. 1998). Whole cell measurements of intact neurons provide important information on the integrative processes and therefore have been exploited in these experiments in a quantitative manner. This paper extends the quantitative analysis of electrotonic structure presented in the previous paper (Saint-Mleux and Moore 2000) to investigate both inward and outward active conductances.

The principal basis of our method is the use of a voltage clamp to isolate the soma from the dendritic tree (Rall 1960). This is possible because the voltage clamp controls the soma potential, effectively isolating parallel dendritic branches and allowing their membrane potentials to respond accordingly. This behavior is responsible for the well known space-clamp problem of neurons with dendritic trees (Rall and Segev 1985; Spruston et al. 1993, 1994; Stuart and Spruston 1998) and means that the measured somatic voltage-clamp responses cannot be analyzed solely with independent parallel ionic currents. The response of the clamp is composed of both somatic membrane currents and most importantly, currents caused by the nonclamped dendritic regions. The channel kinetics of the dendritic membrane determine the unclamped membrane potential responses that in turn produce a somatic voltage-clamp current. It is precisely this current that can be analyzed for dendritic membrane properties. The interpretation of these data requires detailed knowledge of the electrotonic properties to evaluate the active conductances in the soma and dendritic cable.

A whole cell clamp in both voltage- and current-clamp modes was used to constrain more tightly membrane parameters than either method alone. In contrast to the voltage clamp, the constant current stimulus induces a “voltage response” from both the somatic and dendritic regions. The potential profiles of the soma and dendritic membranes are radically different in these two cases and provide a sensitive way to obtain the best parameters for a particular model (Clements and Redmann 1989). Thus a quantitative analysis of the two re-
sponses provides a way to separate and analyze the membrane properties of dendritic and somatic regions.

Dendritic and somatic membrane properties were used to reconstruct the action potential behavior and quantitatively predict the dependence of neuronal firing properties on electrotoneic structure. Finally, this analytic and theoretical approach provides a new quantitative method to investigate the pharmacological properties of neurotransmitter receptors on dendrites (Moore et al. 1999) and their role in the neural network behavior (Dale 1995b; Marder and Calabrese 1996; Prime et al. 1999; Roberts et al. 1995; Tabak and Moore 1998) of spinal cord circuits that are involved in the more complex locomotor patterns of Xenopus larvae.

METHODS

Experimental procedure

Current- and voltage-clamp measurements were made in both the time and frequency domains using methods and analyses identical to those described in the previous paper (Saint-Mleux and Moore 2000). The photomicrograph of Fig. 1 illustrates presumed interneurons that have been separated by overstretching the half spinal cord preparation after the measurements were made. As described previously, both time and frequency domain data were analyzed simultaneously. To quantitatively describe these two data types, it is important to measure them at the same final membrane potential, V(t). This can be best achieved by obtaining all the data during the same step-clamp stimulus. Finally, each neuron was characterized by four types of measurements: real-time constant current and voltage clamp, each of which had a corresponding steady-state frequency (f) domain (white noise) determination: impedance, Z(f), and admittance, Y(f), respectively.

The stimulus protocol for both current- and voltage-clamp measurements was a command step to elicit real time nonlinear kinetic responses followed by a low-amplitude sum of sines, which was superimposed on the preceding step, to obtain frequency domain linear kinetic behavior. The sum of sines stimuli and responses were Fourier analyzed to determine point impedance functions given as the ratio of output to input frequency domain functions. Thus each current- or voltage-clamp step consisted of a nonlinear voltage or current response, respectively, as well as a correspond-

ing linear frequency domain point impedance or admittance function. Finally, the neuronal model obtained from the analysis developed in the following text was required to be consistent with the four types of data.

Space clamp issues

A spatially uniform voltage clamp of a cell is clearly the preferred method for analyzing voltage-dependent membrane properties. Unfortunately, real neurons cannot be space clamped because of their dendritic structure (Major et al. 1993, 1994; Rall and Segev 1985; Spruston et al. 1993). A perfect somatic voltage clamp records somatic currents that originate from the cell body and unclamped dendritic regions. Alternatively, the current clamp injects a constant current into the soma that distributes itself between the soma and the dendritic tree. The transient potential responses for these two types of experiments is clearly different but can be modeled exactly by the appropriate cable equations. A comparison of such a model in both current and voltage clamp (Clements and Redman 1989; Müller and Lux 1993) provides a good test of the accuracy of the model, especially if voltage-dependent conductances are activated. If the neuronal model with its dendritic tree is incorrect, it will not be able to describe both the voltage- and current-clamp data because the dendritic potential responses for the two modes are so different. On the other hand, a good description of both clamp modes provides strong evidence that the cable model can provide an adequate description of the total neuron.

Figure 2 illustrates the essence of our experimental approach where a voltage clamp of the soma leads to potential responses in the dendritic compartments that resemble the measured somatic current. For comparison, the active membrane current generated by the somatic compartment is shown to be significantly smaller than the dendritic effect. By contrast, the response of the same neuronal model to a constant current shows a nearly synchronous response for the somatic and dendritic compartments. It can be seen from these simulations that the similarity between the somatic voltage-clamp currents and the dendritic membrane potential transients indicates that the somatic voltage-clamp current contains significant information about dendritic membrane behavior. This analysis is dependent on an accurate assessment of the electrotonic structure that is obtained readily in the frequency domain.
The quantitative analysis in this paper was done on the neurons of the previous paper, which are identified by the same names in Tables 2–4 (see Tables 4 and 5 of preceding paper). Data over a range of membrane potentials were collected with and without using TTX during internal perfusion of potassium gluconate or cesium sulfate. The basic strategy was to analyze the properties of individual neurons in a sequential manner such that fitted parameters were fixed before proceeding to the next procedure, namely the passive electrotonic structure [hyponym] of the minimal models used here (Bhalla and Bower 1993). Multiple nonunique fits were possible using either voltage or current clamp alone; however, only one set of parameters could be found if all four data types were used. Only three of the data sets actually were needed because the frequency domain results for the voltage and current clamp were required to be consistent as a criterion for adequate instrumentation.

R E S U L T S

The data and figures presented in this section are the result of an extensive analysis of voltage-clamp data near the resting potential to accurately determine electrotonic parameters and both voltage- and current-clamp experiments over a wider range of membrane potentials. As pointed out in the companion paper (Saint-Mleux and Moore 2000), this requires a determination of the adequacy of different dendritic models with regard to numbers of compartments and steady-state errors that can occur due to the potential profile along the dendritic tree. The quantitative analysis in this paper was done on the neurons of the previous paper, which are identified by the same names in Tables 2–4 (see Tables 4 and 5 of preceding paper). Data over a range of membrane potentials were collected with and without using TTX during internal perfusion of potassium gluconate or cesium sulfate. The basic strategy was to analyze the properties of individual neurons in a sequential manner such that fitted parameters were fixed before proceeding to the next procedure, namely the passive electrotonic structure [hyponym] of the minimal models used here (Bhalla and Bower 1993). Multiple nonunique fits were possible using either voltage or current clamp alone; however, only one set of parameters could be found if all four data types were used. Only three of the data sets actually were needed because the frequency domain results for the voltage and current clamp were required to be consistent as a criterion for adequate instrumentation.

The model formulations are identical to those of previous paper (Saint-Mleux and Moore 2000) In this paper, the maximum value of a voltage-dependent conductance, represents potassium (gK, gKc, or gKc2), calcium (gCa), and sodium (gNa) conductances, where the unitless, voltage-dependent variable, x, becomes n or q, s, and m, respectively (see APPENDIX of companion paper). The conductance, gK, is the normal fast potassium conductance, gKc, is a slow potassium conductance possibly calcium ion dependent, and gKc2, is a potassium-cesium conductance having a reversal potential, VKc2, where potassium ions carry inward currents and predominantly cesium ions carry outward currents. The activation variables, m and s, were assumed to be at their steady-state values because the sampling frequency was too slow to measure the activation time constants. The term, h, was only used with the sodium conductance to described inactivation, gNa*m/h*(V - VNa).

The use of the full nonlinear differential equations for parameter estimation is far more difficult than curve fitting voltage-clamp transients of single compartments; however, it is absolutely essential because of the dendritic structures. We have used a modification of the Hodgkin-Huxley (Hodgkin and Huxley 1952) kinetic model (see Borg-Graham 1991; Murphey et al. 1995) that does not use a power model for the voltage-dependent variables. This choice was made for two reasons, a dendritic structure masks the delay of the voltage-clamp transients needed to determine the power function and the general form of the linearized equations is unaffected by the power. A 500-Hz band was chosen to obtain the best compromise between resolution and sampling frequency that was also sensitive to the activation of the voltage-dependent conductances. Our goal is to obtain the minimal model that will fit all the data, namely the nonlinear and linear responses.

The estimation methods, as previously described (Murphey et al. 1995, 1996), were applied initially to the passive data for an estimation of the electrode and electrotonic parameters (Saint-Mleux and Moore 2000). Using fixed passive parameters, the data taken at depolarized membrane potentials were fitted simultaneously in the frequency and time domains with the linear analytic model and a three-dendritic nonlinear compartmental model, respectively. The adequacy of both the frequency and time domain fits was evaluated using a model with 30 dendritic compartments (Bush and Sejnowski 1993; Saint-Mleux and Moore 2000). If necessary, 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).

It should be emphasized that consistent fits of both the constant current- and voltage-clamp data are essential to achieve the best fits of the minimal models used here (Bhalla and Bower 1993). Multiple nonunique fits were possible using either voltage or current clamp alone; however, only one set of parameters could be found if all four data types were used. Only three of the data sets actually were needed because the frequency domain results for the voltage and current clamp were required to be consistent as a criterion for adequate instrumentation.
TTX, calcium and cesium conductances with internal cesium ions in the presence of TTX, and finally sodium and calcium conductances that were determined at subthreshold depolarizations with and without internal potassium ions. All procedures except for the analysis of the sodium conductance were done in the presence of TTX. Internal cesium ions were used to block the potassium conductances to more effectively measure inward sodium and calcium currents. This approach limits the number of estimated parameters for a given experimental condition as well as providing a partial separation of the ionic conductances by both pharmacological and voltage modulation of the individual levels of activation. The result of this analysis provides an experimentally determined model that nevertheless is dependent on some theoretical assumptions. As pointed out by others, such "parameter estimation" should be done with care on as few variables as possible (Dale and Kuenzi 1997; Tabak and Moore 1998).

Voltage-clamp I-V curves

In the companion paper (Saint-Mleux and Moore 2000), it was found that neurons with larger dendritic areas and shorter electrotonic lengths (L) show more accommodation (Type B) than those with smaller areas and larger values of L (Type A). It was also shown that Type B neurons have a highly rectifying current-voltage (I-V) curve. Although the values of the potassium conductance kinetic parameters were similar, the mean slope of the activation curve versus potential (s
A
) was greater in Type B than Type A neurons. This type of voltage dependence tends to cause a more abrupt increase in the current near the half-activation potential and correspondingly leads to a sharp transition in an I-V curve. In addition to the effect of outward potassium currents on the rectification of the I-V curve, both sodium and calcium ions carry steady-state inward currents that counterbalance or override the outward currents.

The voltage clamp is necessary to observe inward currents, which in turn leads to an I-V curve that has a negative slope, usually referred to as a negative conductance. Therefore I-V curves were measured under voltage-clamp conditions because negative slopes cannot be observed in a constant current experiment. Figure 3 illustrates that voltage clamp I-V curves for Type A and B neurons are in dramatic contrast, such that the slope of some Type B cells is zero or possibly negative at potentials that show the maximum rectification. Figure 3B superimposes the fitted model with its parameters and data for a Type B neuron (97J30A) showing that a lower minimum (negative) slope was found in voltage versus current clamp. These results support the hypothesis that steady-state negative conductances are contributing to the I-V curve of these neurons (see following text).

TTX-sensitive negative conductance (g
Na
)

The measurement of sodium and calcium inward currents is best done if the internal potassium ions are replaced by cesium. Figure 4 illustrates the time required to exchange by passive diffusion the normal potassium concentration with cesium ions by observing the consequential changes in the falling phase of the action potential. The decrease in the potassium conductance leads to multiple action potentials even for short-duration pulses. Finally, after nearly complete removal of K⁺, the neuron remains depolarized unless a hyperpolarizing current is injected.

Although inward currents are easily observed in voltage-clamped cesium perfused neurons, their analysis is especially difficult in dendritic structures because of the instabilities that occur in step-clamp measurements. In general, it is not possible to control the sodium action potentials in a somatic voltage clamp; however, subthreshold TTX-sensitive responses can be used to estimate kinetic parameters. As discussed in the preceding text, current-clamp data provided further constraints on the parameter values, which then led to a set of self-consistent parameters for the sodium conductance. In addition, simulations were carried out to explore the sensitivity of the voltage-dependent parameters on action potential behavior (see following text).

Figure 5, A and B, illustrates inward currents associated with marked negative phase functions at low frequencies for −55, −50, and −30 mV, despite an uncontrolled spike at −30 mV (see Fig. 5A, *). The inward currents and low frequency negative phases at −50 and −55 mV were blocked by 1 μM TTX (Fig. 5C and D) but not at −30 mV. The absence of a TTX-insensitive low-frequency negative phase function at −50 mV (Fig. 5D) and its presence at −30 mV is consistent with a sodium conductance that has a threshold higher than the sodium conductance.

The analysis of the sodium conductance was done after the presumed calcium (see following text) and potassium conductance parameters had been determined and fixed. Tables 1 and 3 show the estimated parameters for the activation and inactivation of the sodium conductance of the Type A neurons. The activation variables of both g
Na
 and g
Ca
 were assumed to be in

![Fig. 3. I-V curves for Type I and II neurons under voltage clamp. A: current-voltage (I-V) curves from voltage-clamp data for 4 Type I, A neurons (repetitive firing behavior, ⊕) and 3 Type II, B neurons (accommodating firing patterns; ●). Type B neuron (97J20B) in which no firing pattern was measured. I-V curves were made from the difference in average values of the current 100 ms before the step and the last 100 ms of the step clamp, just before the beginning of the white noise signal. B: current-voltage (I-V) curves for both current (_adc) and voltage (ADC) clamp from the neuron 97J30A show a greater rectification in voltage clamp. ● show the same neuron in A. Superimposed lines in B are the model predictions for this neuron based on parameters given in the tables of this and the preceding paper.](http://jn.physiology.org/doi/10.1152/jn.1997.278.8.1384)
a steady state because the activation time constants are too rapid for our sampling interval. The sodium conductance of Type B neurons was generally not measured because of the difficulties of obtaining controlled responses from a somatic voltage clamp of neurons that had a relatively large electro-tonic area, A (see Fig. 5A for one neuron).

**TTX-insensitive inward conductance** ($g_{Ca}$)

In Type A neurons, inward currents such as calcium currents ($I_{Ca}$), are overwhelmed by the potassium conductance and difficult to measure. However, replacement of intracellular potassium with cesium ions shows a TTX-insensitive inward conductance in both types. Figure 5, C and D, illustrates that these data are well described by one noninactivating putative calcium conductance ($g_{Ca}$). The voltage-clamp current at −30 mV is less positive than those observed at the smaller voltage clamp steps to −55 and −50 mV. Thus an inward current is likely to have been activated as shown by the negative phase function in Fig. 5D (−30 mV). A negative phase function at low frequencies is an extremely sensitive indication of an inward current and can be observed in the presence of a net outward current. This occurs because the negative phase is a manifestation of a negative slope conductance that may or may not be associated with a net inward current.

The mean half-activation potential of the Type A putative calcium conductance was −21 mV (Table 2), which is consistent with a high-threshold calcium conductance (Gu and Spitzer 1993; Spitzer 1991). This conductance was subject to rundown; however, its kinetic properties did not change with time. A small outward current was observed in most of the cesium perfused neurons and probably represents an imperfect selectivity of the potassium channel ($g_{K_2}$). The parameters of this conductance, given in Tables 2 and 3 as $g_{K_2}$, show a relative low half-activation potential and most likely represent cesium and potassium ions passing through a slow potassium channel. A net inward TTX-insensitive current is shown for a Type B neuron in Fig. 6A. The frequency domain curves show marked negative phase functions (Fig. 6B) that have been fitted with a noninactivating calcium conductance and $g_{K_2}$. The calcium conductance kinetic parameters for Type B neurons given in Table 2 show a half-activation potential of −31 mV, which is more negative than found for Type A neurons.

**Constant current kinetics**

We have emphasized the voltage-clamp data in the preceding analysis because the observation of net inward currents provides convincing evidence of both sodium and calcium conductances. Nevertheless the final analysis of all aspects of these measurements always includes a confirmation of current-clamp data. In some instances, the current-clamp responses are more sensitive indicators of underlying conductances than the voltage clamp. One such case is illustrated by Fig. 7 in which regenerative oscillatory responses were observed. This neuron in TTX was fitted with a model containing one inward ($g_{Ca}$) and two outward conductances ($g_K$, $g_{K_2}$). The inward putative calcium conductance ($g_{Ca}$) was responsible for the inflection on the rising phase, and a slow outward conductance ($g_K$) caused the oscillatory response seen at the initial depolarizations. The damped overshooting constant current responses at the more depolarized levels were described by a fast potassium conductance ($gK$). It was virtually impossible to fit these responses with only one potassium conductance. The $g_K$, was not re-
Type A neurons are quite similar; however, the determination of a half-activation potential, $v_a = -31$ mV, for Type B neurons suggests a relatively low-threshold calcium conductance (Gu and Spitzer 1993; Sun and Dale 1998b). The determination of a low-threshold, presumed calcium conductance for Type B neurons in K gluconate neurons implies that the potassium conductances were less activated at lower depolarizations than Type A neurons.

![Graphs](image)

**FIG. 5.** Sodium and calcium conductances with internal cesium ions. Sodium conductance was estimated after the calcium conductance had been estimated in TTX. Sodium conductance was estimated after the calcium conductance had been estimated in TTX. Sodium conductance was estimated after the calcium conductance had been estimated in TTX.

![Graphs](image)

**TABLE 2. Calcium conductance parameters of Type A and B neurons**

<table>
<thead>
<tr>
<th></th>
<th>Type A</th>
<th>Type B</th>
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<tbody>
<tr>
<td>$g_{Ca}$, pS</td>
<td>51.6 ± 23.0</td>
<td>47.2 ± 79.7</td>
</tr>
<tr>
<td>$v_a$, mV</td>
<td>-19.81 ± 5.68</td>
<td>-31.05 ± 9.37</td>
</tr>
<tr>
<td>$r_a$, mV$^{-1}$</td>
<td>0.25 ± 0.10</td>
<td>0.078 ± 0.069</td>
</tr>
<tr>
<td>$s_a$, mV$^{-1}$</td>
<td>-0.02 ± 0.12</td>
<td>-0.126 ± 0.098</td>
</tr>
<tr>
<td>$g_{KCs}$, pS</td>
<td>94.7 ± 11.7</td>
<td>—</td>
</tr>
<tr>
<td>$v_{KCs}$, mV</td>
<td>-26.7 ± 14.1</td>
<td>—</td>
</tr>
<tr>
<td>$r_{KCs}$, mV$^{-1}$</td>
<td>0.039 ± 0.001</td>
<td>—</td>
</tr>
<tr>
<td>$s_{KCs}$, mV$^{-1}$</td>
<td>193 ± 91</td>
<td>—</td>
</tr>
<tr>
<td>$g_{Kleak}$, mV$^{-1}$</td>
<td>-1.12 ± 0.04</td>
<td>—</td>
</tr>
<tr>
<td>$v_{Kleak}$, mV</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$r_{Kleak}$, ms</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$s_{Kleak}$, ms</td>
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</table>

Values are means ± SD.

**TABLE 1. Sodium conductance parameters**

<table>
<thead>
<tr>
<th></th>
<th>Type A</th>
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<tbody>
<tr>
<td>$g_{Na}$, nS</td>
<td>3.45 ± 3.39</td>
</tr>
<tr>
<td>$v_m$, mV</td>
<td>-21.63 ± 6.53</td>
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<tr>
<td>$r_m$, mV$^{-1}$</td>
<td>0.053 ± 0.023</td>
</tr>
<tr>
<td>$s_m$, mV$^{-1}$</td>
<td>0.037 ± 0.021</td>
</tr>
<tr>
<td>$v_h$, mV</td>
<td>-47.16 ± 1.71</td>
</tr>
<tr>
<td>$r_h$, mV$^{-1}$</td>
<td>0.054 ± 0.028</td>
</tr>
<tr>
<td>$t_h$, ms</td>
<td>147 ± 133</td>
</tr>
<tr>
<td>$r_h$, mV$^{-1}$</td>
<td>0.049 ± 0.002</td>
</tr>
</tbody>
</table>

Values are means ± SD.
neurons. This is consistent with a more shallow activation curve ($s_n = 0.04$) for Type A than the steeper slope, $s_n = 0.09$, of the fast $g_K$ activation curve of Type B neurons, despite similar half-activation potentials ($v_n = -10$ to $-15$ mV) for both types (see Table 3 of preceding paper). The additional Type B potassium conductance, $g_K^{2}$, is two orders of magnitude smaller than the fast $g_K$ and has an activation threshold, $v_q = -39$ mV, that is significantly more negative (see Table 2) than found for the $g_K$ of Type A or B neurons (Saint-Mleux and Moore 2000).

### Steady-state potential profile

The current-clamp data were well fitted by the three-dendritic compartmental model for the initial depolarizations; however, discrepancies in the frequency domain occur at the more depolarized levels (Fig. 7, B and C). The magnitude of the impedance of a 30-compartmental model was significantly greater (Fig. 7D) than given by the analytic model having the same parameter values (Fig. 7C). At the resting potential, the two versions of the same model, compartmental and analytic, give nearly identical results. This result is an example of a steady-state dendritic potential profile that leads to a decreased activation of the potassium conductance in the peripheral regions of the dendrite. When an analytic model is used to describe the frequency domain, it is assumed that the voltage-dependent conductances are activated identically in all compartments, thus leading to a lower impedance magnitude (greater activation of $g_K$) than actually measured. It is rather remarkable that the parameters, which give a good fit only at potentials more negative than $-30$ mV with the analytic model, provide an excellent prediction at all potentials using a 30-compartmental dendritic model (Fig. 7D).

#### FIG. 6. Calcium conductance of a Type B neuron in presence of TTX. Currents were fitted with one noninactivating inward conductance and an outward conductance. Model parameters are $C_{soma} = 2.4$ pF, $L = 0.13; A = 6.03; g_{soma} = 0.013$ nS; $V_{h} = -25.6; g_{Ca} = 0.024$ nS; $v = -30.7$ mV; $s_n = 0.044$ mV$^{-1}; r_s = 0.04 mV^{-1}; v_C = 100$ mV; $g_{K} = 0.034$ nS; $v_q = -33.2$ mV; $s_n = 0.02 mV^{-1}; t_q = 272$ ms; $v_q = -0.1$ mV$^{-1};$ and $V_{K} = -90$ mV. Data taken with the same voltage clamp step for the time (A) and frequency (B) domains. Note that the $-33$ mV steady-state current is between the step-clamp currents associated with $-13$ and $-23$ mV. Phase function at both $-33$ and $-23$ mV remains negative over the entire frequency range, demonstrating the presence of a negative slope conductance. Neuron 97K17C, Type B as in Fig. 4.
The passive conductance of the dendritic cable (see Eq. A14 of Saint Mleux and Moore 2000), $g_{dendrite}$, is given by the expression, $(A/L)^2 g_{soma} \tanh(L)$, where $g_{soma}$ is the reciprocal of the passive soma resistance, $A$ is the dendritic to soma area ratio, and $L$ is the electrotonic length. Thus the dendritic structure is composed of three variables that were shown to form a cluster of parameter values that allowed the definition of two groups of neurons, Type A and Type B, presumably with different cable morphologies (Saint-Mleux and Moore 2000). Because the Type B neuron shows relative high values of $A$ and low $L$ values, the ratio, $A/L$, provides a single parameter to distinguish Type A and B neurons. Figure 8 illustrates at least two clusters of neurons in a three-dimensional plot of $g_{dendrite}$, $A$, and $L$. One group (★ and ●), identified as Type B has $A/L > 24$ and $g_{dendrite} < 500 \mu S$, and the other, Type A (○ and ●), consists of the remaining neurons that also have a broad range of dendritic area ratios that tend to cluster near 4. The advantage of the coordinates of Fig. 8 is ability to separate the neurons by one plane, which was not the case for plots with $g_{soma}$, $A$, and $L$ as axes. The finding that $A/L$ rather than $g_{dendrite}$ is more discriminatory suggests that there is a greater difference in the structural parameters, $A$ and $L$, compared with other passive membrane properties. In Fig. 8, ★ and ● represent instances where the firing pattern of the neuron was measured, which in all cases showed a correspondence between repetitive firing for Type A and significant accommodation for Type B.

A computational analysis (Bush and Sejnowski 1993; Mainen et al. 1995) was done using the parameters associated with each type of neuron to compare the predicted and observed action potentials. Type I repetitive firing patterns were
obtained when simulations were done with the parameter values of Type A neurons. In these computations, the mean passive and active parameters of Type A neurons were used with the exception that the maximum fast potassium conductance was increased. The slow potassium conductance in this model was assumed to be identical with \( g_{K_t} \), which is similar to the \( g_{K_t} \) parameters of Type B neurons (see Table 2 and legend of Fig. 9). The need for an increase in the fast potassium conductance probably represents an underestimate of our maximum conductance in these experiments because they were done at relative low membrane depolarizations to characterize the cells near their resting potential. Simulations of Type II behavior were also done with the measured Type B parameters as given in the legend of Fig. 10.

The Type A constant current simulations (Fig. 9, A–C) show increased numbers and frequencies of action potentials with current intensity as was observed for Type I neurons (Saint Mleux and Moore 2000). A clear rectification was obtained for the model with Type A parameters (Fig. 9D) that corresponds well with that observed for the Type I neurons (Fig. 3A). The action potential frequency of the Type A model also could be increased by decreasing the slow potassium conductance (Fig. 9E) with minimal changes in the \( I-V \) curve.

Figure 10 illustrates that a neuronal model derived from the averaged data of Type B neurons (Tables 1 and 2) gives a single action potential response to 0.02-nA maintained constant current if the \( g_{Na} \) is one-half that of Type A neurons and the half-activation potential of the sodium conductance, \( V_{m0} \), is \(-28 \text{ mV}\) (Fig. 10A). This single response can be converted to a rapidly adapting train of action potentials by slightly shifting the \( V_m \) by 3 to \(-25 \text{ mV}\), as illustrated in Fig. 10B. Under these conditions, reducing the current to 0.01 nA (Fig. 10C) elicits two action potentials after which the potential decays nearly to the resting value despite the maintenance of the constant current. This behavior is similar to that observed in Type II neurons (Saint Mleux and Moore 2000). The correspondence of the Type B model with the Type II neurons suggests that neurons with relatively large dendritic membrane areas show

**FIG. 8.** Electrotonic parameters of Type A and B neurons. Three-dimensional plot was constructed by plotting the dendritic conductance \( (g_{dendrite}) \), the electrotonic length \( (L) \), and ratio \((A/L)\) of the dendritic surface to soma area ratio \((A)\) and \(L\). Type A neurons (○ and ●) are defined with \( A/L < 24 \) and Type B (● and ○) > 24. ● and ○ were neurons in which firing patterns were measured. In each case, the Type A neurons fired repetitively and the Type B showed strong accommodation.

**FIG. 9.** Type A neuronal model simulations. A: threshold and hyperpolarizing responses with ±20-pA constant current stimulation; B: multiple action potential responses to 30-pA stimulation that are not sustained; C: sustained firing of action potentials with a 60-pA maintained stimulation; D: current-voltage \((I-V)\) relationships showing a marked rectification similar to the Type I neurons of Fig. 3. E: increased frequency of action potentials for 30 pA where the slow potassium conductance, \( g_{K_s} \), was reduced to 0. Type A model’s passive parameters are \( c_{m,m} = 2.9 \text{ pF}; L = 0.34; A = 4.2; g_{Na,m} = 0.125 \text{ nS}; V_{cach} = -59 \text{ mV}; \) Active conductance parameters are \( g_K = 7.2 \text{ nS}; v_{th} = -15 \text{ mV}; \Delta t = 0.04 \text{ mV}^{-1}; \Delta t = 8 \text{ ms}; \gamma = -0.02 \text{ mV}^{-1}; g_{K_s} = 0.05 \text{ nS}; v_{th} = -33 \text{ mV}; \alpha = 0.06 \text{ mV}^{-1}; \tau = 0.9 \text{ s}; \tau = -0.15 \text{ mV}^{-1}; \) and \( V_{K} = -90 \text{ mV} \). Calcium conductance parameters are \( g_{Ca} = 0.11 \text{ nS}; v_{th} = -18 \text{ mV}; \alpha = 0.04 \text{ mV}^{-1}; \gamma = -0.12 \text{ mV}^{-1}; \) and \( V_{Ca} = 100 \text{ mV} \). Sodium conductance parameters are \( g_{Na} = 2.4 \text{ nS}; v_{th} = -25 \text{ mV}; \gamma = 0.06 \text{ mV}^{-1}; \tau = -0.007 \text{ mV}^{-1}; \) and \( v_{in} = -51 \text{ mV}; \gamma = -0.04 \text{ mV}^{-1}; \tau = 0.15 \text{ ms}; \gamma = 0.05 \text{ mV}^{-1}; \) and \( V_{Na} = 150 \text{ mV} \).
neurons may be partly due to electrotonic differences, a small shift of the sodium conductance activation is sufficient to produce the observed Type II behavior in the Type B model. The rectification of the I-V plot is enhanced by the sodium and calcium conductances that are both responsible for the negative slope under voltage clamp and flattening under constant current. An increased negative slope is seen with the simulation using \( v_m = -28 \text{ mV} \) due to the activation of a negative conductance at a more negative potential than occurs for \( v_m = -25 \text{ mV} \). A negative slope in an I-V plot cannot be observed under constant current conditions because a steady-state potential cannot be maintained in such an unstable region. The corresponding I-V plots for current- or voltage-clamp conditions of the Type A model are essentially identical because no unstable negative slopes were observed under voltage clamp.

Current voltage curves measured under soma voltage clamp illustrate the marked differences in rectification of Type I and II neurons (Fig. 3A). Current voltage curves for Type I neurons appear identical for voltage (Fig. 3A) and current clamp (Saint Mleux and Moore 2000); however, a small increase in the rectification during a voltage clamp could be observed in Type II neurons, as illustrated in Fig. 3B. Thus the Type A and B model simulations are consistent with the experimentally observed I-V curves for the Type I and II neurons.

**FIG. 10.** Type B neuronal model simulations. A: single action potential response of Type B neuron with \( v_m = -28 \text{ mV} \). B: multiple firing pattern with accommodation of Type B neuron with \( v_m = -25 \text{ mV} \). C: doublet response of Type B neuron with lower current level using parameters identical to those in B. Superimposed I-V plots of D show constant current (—) vs. voltage-clamp determinations for the 2 values of \( v_m \) used in the simulations of A and B, namely \(-28 \) and \(-25 \text{ mV} \). A greater rectification was observed for \( v_m = -28 \text{ mV} \) because the inward sodium conductance was activated at a less depolarized potential. Voltage clamp allows the observation of a negative slope in the I-V plot, which cannot occur with constant current determinations because of instabilities due to negative currents. Type B model’s parameters are \( C_m = 2.6 \text{ pF}; L = 0.21 ; A = 6.7 ; g_{ soma} = 0.034 \text{ nS}; V_{ leak} = -53 \text{ mV} ; g_{ shunt} = 0.34 \text{ nS}; V_{ shunt} = -70 \text{ mV} \) for the voltage-independent parameters. \( g_{ shunt} \) is a second leakage conductance present only in the soma in addition to \( g_{ soma} \). Active conductance parameters are \( g_N = 2.4 \text{ nS}; V_N = -15 \text{ mV} ; s_N = 0.09 \text{ mV}^{-1} \cdot t_N = 15 \text{ ms}; \eta_N = -0.03 \text{ mV}^{-1} \cdot g_K = 0.03 \text{ nS}; V_K = -39 \text{ mV} ; s_K = 0.05 \text{ mV}^{-1} \cdot t_K = 0.4 \text{ s}; \eta_K = -0.09 \text{ mV}^{-1} \cdot g_{ Ca} = 0.01 \text{ nS}; V_{ Ca} = -35 \text{ mV} ; s_{ Ca} = 0.09 \text{ mV}^{-1} \cdot t_{ Ca} = -0.11 \text{ mV}^{-1} \). Sodium conductance parameters are identical except that \( g_{ Na} \) was reduced by one-half.

**DISCUSSION**

In addition to the use of frequency domain functions for an analysis of electrotonic structure, such functions also provide a complete description of the dynamic impedance of neurons at subthreshold membrane potentials. The linear neuronal models determined from the measurements in this paper include all of the active conductances (Surkis et al. 1998) and can be used to predict small signal synaptic events. The measurements themselves are single point impedance functions (data) that can predict responses to small currents at somatic locations. Current inputs at peripheral regions also can be computed from the derived models with two point transfer functions (model). The main limitation of this approach for synaptic events is the requirement of a quasi steady state condition; however this is often the case for incoming synaptic phenomena.

Anomalous impedance increase during activation of negative conductances

In addition to the negative phase functions, an activation of the sodium and/or calcium conductances causes increased impedance magnitudes at low frequencies. Figures 5 and 6 show impedance functions that have maximal low-frequency magnitudes at membrane potentials of \(-55 \) to \(-50 \text{ mV} \) for a cesium perfused neuron and \(-23 \text{ mV} \) in the presence of TTX. The former represents the effects of both \( g_{ Na} \) and \( g_{ Ca} \) in a Type B neuron and the latter is due to a presumed \( g_{ Ca} \) in a Type B neuron. These effects have been enhanced by the lack of a significant potassium conductance; however, this can be expected to occur normally and play a role in the integration of synaptic events. Such increased impedances decrease effective electrotonic lengths (Moore et al. 1999) at relatively low frequencies and provide a way to control somatic responses to dendritic synaptic inputs (Buchanan et al. 1992; Moore et al. 1994, 1995; Stuart and Sakmann 1995). This phenomenon
occurs because of a dynamic decrease in the effective electrotonic length that can be caused by the activation of any negative conductance.

The enhanced negative conductance seen during cesium perfusion may be caused partially by some inward potassium current passing through an A-current channel. A cesium-insensitive potassium A current has been demonstrated in mammalian central neurons (Sanchez et al. 1998), which is inward at potentials more negative than $-35$ mV. We have not been able to fit the cesium data with $V_{KCS} = -35$ mV; however, good fits are possible with $V_{KCS} = -50$ to $-90$ mV. In all cases an inward calcium current was required to describe the data. We occasionally have observed strongly inactivating A-type currents in cesium-perfused neurons that show a marked negative slope conductance (unpublished data). These currents were always outward and much larger than the inward calcium currents.

**Comparison of embryonic and larval neurons**

Ionic conductances of spinal neurons from the *Xenopus* preparation have been quantitatively analyzed in isolation at stages 37/38 (Dale 1995a; Harris et al. 1988; Kuenzi and Dale 1998) and in culture or reduced preparations at earlier stages (Desarmenien et al. 1993; Lockery and Spitzer 1988). The mathematical descriptions in these investigations were single-compartment models of the soma because dendritic structures are minimal at the earlier stages. The neurons investigated in this paper represent later developmental stages, 42–47, and have been measured within an essentially intact spinal cord. Although the theoretical and experimental conditions of these different preparations are not identical, some comparison of the derived experimental parameters is possible. In addition to cell isolation, an additional important difference between our experiments and those of Dale and his coworkers (Dale 1995a; Sun and Dale 1998a,b) is the external calcium concentration. The 10 mM calcium levels used by Dale (1995a) are likely to shift all activation curves to more depolarized values than would be observed at 1 mM calcium ions used in our experiments. This effect also would diminish the differences that appear to exist between early and late stages of development that are indicated in the following text. A further point is that high calcium levels act like a hyperpolarization that tends to enhance the delay in the turning on of voltage-dependent conductances. This delay was originally modeled by Hodgkin and Huxley (1952) by the power on the voltage-dependent potassium conductance. Although such a power function causes a difference between the half-activation potential of the conductance and the voltage-dependent variable ($x_n$), we generally have made our comparisons with the conductance rather than $x_n$.

The current-voltage curves of the embryo (Dale 1995a) suggest that the fast and slow potassium slope conductances have reached their maximum values by 0 mV and are half-activated between 0 and $-20$ mV. Our analysis at later stages gave similar results for the half-activation potentials ($v_n = -10$ to $-15$ mV) of the fast potassium conductance, $g_{Ks}$ (Saint Mieux and Moore 2000) and a more negative $v_n$ of $-39$ mV for $g_{Ks}$, the slow potassium conductance. Pharmacological studies on isolated cells at stage 37/38 (Kuenzi and Dale 1998) have shown that dendrotoxins significantly block the slow potassium currents and correspondingly enhance repetitive firing. This result was in contrast to the reduction by catechol of the fast potassium current, which had relatively minor effects on sustained action potentials; however, isolated neurons from stage 42 show firing properties that are partially dependent on a fast-activating Ca$^{2+}$-dependent K$^+$ current (Sun and Dale 1988a). These measurements suggest that a fast calcium-dependent potassium conductance replaces part of the fast and slow embryonic potassium conductances (Wall and Dale 1993, 1994) without a change in the overall magnitude of the total potassium current (Sun and Dale 1998a). Such developmental changes appear to occur by downregulation of the normal K$^+$ conductances, possibly both fast and slow. This phenomenon could account for the lack of a significant slow $g_{Ks}$ in our voltage-clamp measurements of Type A neurons (Saint Mieux and Moore 2000). It is thus likely that a significant fraction of the potassium currents from intact neurons at stages 42–47 may be due to a fast Ca$^{2+}$-activated K$^+$ current. Furthermore our Ca$^{2+}$-activated potassium currents in 1 mM Ca$^{2+}$ may be low because it has been shown that reducing the Ca$^{2+}$ from 10 to 2 mM reduces the current by 40% (Sun and Dale 1998a).

The maximum calcium currents for isolated embryonic and larval cells occurred $\sim 10$ mV (Dale 1995a; Sun and Dale 1998b), which provides an upper limit for the half-activation of the conductance. The lower limit would be $-10$ mV, the potential for the maximum negative slope conductance (Dale 1995a). Thus the half-activation potential of $-18$ mV for Type A intact larval neurons is more negative than estimated for stage 37/38. The half-activation potential, $v_m \equiv 0$, found at stage 37/38 for the sodium conductance (Dale 1995a) is also less negative than $-22$ mV obtained from Type A larval neurons (Table 1).

Although there are different half-activation potentials for embryo and larval neurons, the potential at the peak values of the time constants are usually very similar. These comparisons are partly model dependent and thus the effect of development on membrane properties needs to be explored more thoroughly. Nevertheless the finding that the half-activation potentials of some ionic conductances observed at later larval stages are at more negative membrane potentials than found for stage 37/38 is consistent with the findings of O‘Dowd et al. (1988) on cultured *Xenopus* neurons at different stages of development (Spitzer 1981). The voltages for half-activation of the steady state variables for the conductances of young versus mature neurons (Lockery and Spitzer 1988) were as follows: potassium: 5 and $-10$ mV; sodium: $-16$ and $-12$ mV; and calcium: 4 and 2 mV, respectively. Although, the sodium conductance showed a 4-mV shift in the opposite direction, the calcium-activated potassium conductance showed a difference similar to the delayed rectifier (Lockery and Spitzer 1988). Thus more mature neurons seem to have lower activation potentials for their voltage-dependent potassium conductances and consequently are likely to possess a wider repertoire of responses such as bursting and repetitive activity.

Our reconstructed action potentials for Type A and B neurons suggest that electrotocnic structure is associated strongly with the neuronal firing properties. Previous simulations of embryonic neurons (Dale 1995b) have shown that repetitive behavior is principally dependent on the slow potassium conductance. Although our slow potassium conductance for larval neurons is relatively small, Fig. 9E shows that reducing it to zero increases the frequency of simulated action potentials in a
manner similar to that found by Dale’s single compartment model of embryonic neurons (Dale 1995b). Decreasing the value of \( L \) had little effect on the firing behavior; however, increasing the dendritic area, \( A \), reduced repetitive firing. Despite this dependence on \( A \), simulations with only the somatic compartments of both Type A and B neurons maintains the appropriate firing pattern. The main difference in the voltage-dependent conductances in our Type A and B neurons is in the parameter, \( s_n \), the slope of the activation curve. Interchanging the \( n \) values converts a Type B model to a repetitive firing neuron and changes the repetitive response of the Type A model to one with plateau oscillations. Thus our model simulations show that repetitive firing is strongly dependent on voltage-dependent potassium conductances and slightly modified by changes in electrotonic structure. Nevertheless the firing patterns are clearly correlated with different electrotonic structures.

Finally, the analysis of these experiments provides a quantitative description of putative spinal interneurons of Xenopus larvae that consists 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 and experimental conditions. Because computational efficiency is an important aspect of neuronal modeling, it is useful to use a minimal number for both data analysis and simulation of network behavior. The criteria developed in this paper provides a means to determine the number of compartments needed for any electrotonic structure with its associated voltage-dependent conductances. The greatest discrepancies occurred in prediction of the voltage-clamp currents. The more nonlinear constant current responses tend to equalize their compartmental potentials due to the presence of active conductances. The use of analytic models avoids compartmental issues; however, errors occur with these models when a large dendritic potential profile leads to an inhomogeneous activation of ionic conductances. Fortunately reasonably good initial estimates of steady-state conductances can be obtained with analytic formulations; however, at large depolarizations the potential profile effect must be taken into account.

In summary, our analysis strategy has addressed the electrode and electrotonic structure by linear analysis and the active properties by a nonlinear analysis of the voltage-dependent behavior expressed through the electrotonic structure. The assumption that the voltage-dependent conductances for sodium, calcium, and potassium ions are distributed uniformly was adequate for the analysis. This method could be extended further to address the spatial distribution of the receptors in more detail (Murphy et al. 1995). The reconstructed action potentials based on all the data reproduced the behavior of two broad distributions of interneurons characterized by their degree of accommodation. Although previous analyses of embryonic neurons suggest that the control of repetitive firing appears to be principally due to a slow potassium current (Dale and Kuenzi 1997), it is clear that electrotonic structure is also an important aspect of this behavior in larval neurons. The equivalence between Types I and II firing patterns and Types A and B neurons shows that this behavior in larval neurons is associated with both the electrotonic structure as well as the specific voltage-dependent conductances. It remains to be determined just how these neurons interact in a network to produce the complicated locomotor patterns characteristic of Xenopus larvae.