Detection of a Membrane Shunt by DC Field Polarization During Intracellular and Whole Cell Recording

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Svirskis, Gytis, Aron Gutman, and Jørn Hounsgaard. Detection of a membrane shunt by DC field polarization during intracellular and whole cell recording. J. Neurophysiol. 77: 579–586, 1997. Lower input resistance with intracellular recording, rather than with whole cell recording, usually has been ascribed to a shunt produced by penetration injury. An alternative explanation is a higher input resistance during whole cell recording due to wash-out of cytoplasmatic substances. We have used neuronal polarization at the onset and termination of an applied electric field for shunt detection. An analytical expression was derived for field-induced polarization in a shunted ohmic cable. When the shunt is negligible, the transient response to a step in DC field decays much faster than the response to current injected through the recording electrode. In the case of a significant shunt an over- and undershoot of the transmembrane potential appear at the shunted end when the field is switched on and off. Over- and undershoot decay with the same slowest time constant as the response to injected current. The results for the cable are generalized for nonuniform fields and arbitrary branching neurons with homogeneous membrane. The field effect was calculated for two reconstructed neurons with different branching pattern. The calculations confirmed the theoretical inferences. The field polarization can be used for shunt detection. The theory was checked experimentally in 18 ventral neurons in transverse slices of the turtle spinal cord. In seven neurons, field-induced undershoots were observed when sharp electrodes were used. This indicates the presence of an injury shunt. In the remaining 11 neurons, however, there were no under- or overshoots, indicating that a shunt is not always induced. When patch electrodes were used, the seal quality was checked by inducing a spike with a strong field stimulus before and after the rupture of the membrane. When the threshold field strength for spike initiation was not changed by membrane rupture, under- and overshoots were not observed. This was taken to indicate a good seal. In such recordings under- and overshoots were observed when a shunt was induced by local application of glycine. The fast and monotonic response to weak field stimulation suggests homogeneous electric properties of the soma-dendritic membrane when active conductances are not recruited. We propose using polarization by weak DC fields to ensure the quality of recordings with sharp and whole cell electrodes and for checking the ohmic homogeneity of the membrane. These controls are particularly important for evaluation of electrotonic parameters.

INTRODUCTION

The neuronal input resistance, $R_{\text{in}}$, during intracellular recording frequently is several times lower than observed with whole cell techniques. This difference usually is ascribed to an impalement injury shunt with a resistance several times smaller than the ‘‘true’’ $R_{\text{in}}$ (Major et al. 1994; Pongracz et al. 1991; Spruston et al. 1994; Staley et al. 1992; Stefani and Steinbach 1969; Thurbon et al. 1994; Ulrich et al. 1994). However, such an interpretation requires additional explanations. One has to explain why the resting potential is almost the same in whole cell and intracellular recordings and why substantial changes in $R_{\text{in}}$ in response to physiological and pharmacological stimuli are present during intracellular recordings (e.g., Barkai et al. 1994; Skydsgaard and Hounsgaard 1994). It is possible that the penetration injury brings about hyperpolarizing currents by activating pumps and ionic channels, e.g., by Ca-dependent mechanisms (Clements and Redman 1989; Pongracz et al. 1991; Staley et al. 1992). However, there is experimental evidence that $\left[\text{Ca}^{2+}\right]$, does not depend on the recording technique in some cases (Al-Mohanna et al. 1994). Therefore one may not discard the explanation that the electrical properties of the cells are changed by whole cell recording due to wash-out of second messengers and other substances (Neher 1992). Indeed, once a whole cell contact is formed, $R_{\text{in}}$ increases with time (Major et al. 1994). It seems, then, that neither recording mode leaves the intrinsic properties of neurons unaffected. Obviously, perforated patch recording is more sparing than ordinary whole cell recording, but it is associated with artificial ionselective conductances and a large access resistance (Jackson 1992; Spruston and Johnston 1992). It is noteworthy that the $R_{\text{in}}$ values are lower with perforated patch recording than with the ordinary whole cell techniques (Major et al. 1994; Spruston et al. 1994).

There are several theoretical and experimental studies devoted to the assessment of membrane shunts (Clements and Redman 1989; Durand 1984; Holmes and Rall 1992a; Iansek and Redman 1973; Kawato 1984; Pongracz et al. 1991; Staley et al. 1992; White et al. 1992, 1994). All these methods employ simulation of the electrophysiological phenomena using off-line multiparameter models and often can not produce unambiguous inference (White et al. 1992). To tackle the disagreement between intracellular and whole cell recordings, one should find a robust way of assessing the shunt resistance, $S$, during experiments or, at least, document the very existence of a tangible shunt.

In the present paper, we present a theory for shunt detection using DC field stimulation and apply and evaluate the theoretical findings in experiments on ventral horn neurons in an in vitro preparation of the spinal cord of the turtle. The paper consists of three main parts: first, the theory for a cable is developed and generalized for an arbitrary branching...
neuron; the second section presents a numerical analysis for two neurons with different branching pattern; finally the theoretical findings are evaluated experimentally in recordings from interneurons and motoneurons in the spinal cord of the turtle.

METHODS

Calculations

For the investigation of field effects, a model neuron was placed in an extracellular potential field with uniform gradient. Only the passive responses were modeled. We have used published reconstructions of two nerve cells.

The first is an interneuron (Fig. 1C) from the stratum pyramidale of the CA1 field in the rat hippocampus (Thurbon et al. 1994). The axon of this cell branches almost symmetrically with respect to the stratum pyramidale. The neuron has just four dendritic trunks with limited branching. The field was oriented in the direction of the apical dendrites in the temporal plane. The dendrites were divided into linear cylindrical segments. For each segment, its angle with respect to the field was determined. The contribution to the soma polarization of the symmetric axon may be neglected (Gutman and Svirskis 1995; Tranchina and Nicholson 1986).

The second cell used in computations is a turtle motoneuron (Fig. 2C in Ruigrok et al. 1984). The diameters, $D$, of the dendritic branches were kept constant between branching points and were derived from the regression curve, $D = 0.55 + 0.53n \mu m$, where $n$ is the number of the terminal branches emerging from the particular branch. The smallest diameter was $1 \mu m$. The field was oriented in the lateral direction and was uniform. The axon was approximately perpendicular to the chosen field direction and therefore not considered in the calculations.

The compartment method (Baginskas et al. 1993; Rall 1989) and Fourier expansion for cylindrical segments were employed to compute the transmembrane potential induced by the field. The Fourier technique is known to generalize stationary computations (Ali-Hassan et al. 1992; Gutman and Svirskis 1995; Tranchina and Nicholson 1986) by replacing real parameter values with complex values (Gutman 1980; Tranchina and Nicholson 1986). The results obtained with these two methods were identical.

Experiments

Transverse sections of the lumbar spinal cord were obtained as described before (Hounsgaard et al. 1988) from turtles (Pseudemys scripta elegans) deeply anesthetized by mebumal (100 mg/kg) injected intraperitoneally. The bath medium contained (in mM) 120 NaCl, 5 KCl, 15 NaHCO$_3$, 20 glucose, 2 MgCl$_2$, and 3 CaCl$_2$. The solution was saturated with 98% O$_2$-2% CO$_2$, to obtain a pH of 7.6 in the recording chamber. 6-cyano-7-nitroquinoxaline-2,3-dione (40 mM; Tocris Neuramin) was applied to block excitatory synaptic potentials. For induction of an artificial shunt, glycine was applied by pressure from a broken micropipette near the recording electrode.

For experiments, a section of the cord, 2 mm thick, glued on to a piece of filter paper, was placed in the recording chamber between two silver-chloride electrodes (the principal scheme of the setup is shown in Fig. 2A) (see also Hounsgaard and Kiehn 1993). The electrodes with a surface area of 12 mm$^2$ were purchased from Clarck Electromedical. Current for field stimulation was delivered by an isolation unit (Isolator 11 from Axon Instruments). The extracellular potential gradient in the tissue was 3–4 mV mm$^{-1}$ 50–200 mm below the surface of the tissue.

For recording field effects, sharp and patch electrodes were pulled from the borosilicate glass tubes with an outer diameter of 1.5 mm and an inner diameter of 0.86 mm. Sharp electrodes were filled with 1.5 M KCl and 0.5 M potassium acetate. Patch electrodes were filled with 125 mM potassium gluconate and 9 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, pH was adjusted to 7.4 with KOH. To reduce noise, 128 or 256 sweeps were averaged on a HIoki digital oscilloscope. The averaged data from the oscilloscope were transferred to an IBM compatible computer through a National Instruments GPIB interface for storage and later analysis. After all measurements were accomplished, the electrode was withdrawn from the cell and the extracellular potential, induced by the same field step stimulus, was recorded, averaged, and

![FIG. 1.](http://jn.physiology.org/)
subtracted from the intracellular potential to get the transmembrane potential. Recordings were obtained from motoneurons and interneurons in the ventral horn. The cell type and health was inferred from the firing properties of the cells (Hounsgaard and Kjaerulf 1992; Hounsgaard et al. 1988). A shunt detection was done only in the cells that were assumed to be healthy.

RESULTS

Theory

Here we take only passive membrane properties into account, so the field is assumed to be too weak to activate potential dependent conductances. The shunt is supposed to be ohmic (Holmes and Rall 1992a) (see DISCUSSION). We are considering only the field induced potential change superimposed on the initial potential distribution. This distribution may be nonuniform due to a shunt. To assess the influence of a shunt on the membrane polarization induced by an electric field, let us first consider a simple case of a cylindrical cable with the shunt at one end. When the cable is embedded in a uniform field directed along the cable, the extracellular potential $U = -Ex$, where $E$ is the strength of the field expressed as the change in potential over one electrotonic length unit, and $x$ is the coordinate along the cable in electrotonic units. The field induces positive transmembrane potentials and charges at the anodic end of the cable and negative potentials and charges at the cathodic end. If the shunt is negligible, the distribution of the negative and positive potentials is symmetrical. As a shunt causes leak of nearby charges, the symmetry is lost. The steady state distribution of the transmembrane potential after a step in DC field is (APPENDIX A)

$$W(x) = E \left[ \frac{\omega}{S} \sinh (x) - \cosh (x - L) \right] / \left[ \sinh (L) + \frac{\omega}{S} \cosh (L) \right]$$

where $L$ is the electrotonic length of the cable, $\omega = (R\rho / \Pi s)^{1/2}$ is the characteristic resistance of the cable, $R$ is the specific membrane resistance, $\rho$ is the specific cytoplasmic resistance, $\Pi$ is the perimeter, $s$ is the cross section area of the cable, and $S$ is the shunt resistance.

The transient solution can be derived employing the technique of variable separation (Rall 1969, 1989). The full solution describing the field effect after switch-on of the DC field is the following (APPENDIX B)

$$W(x, t) = W(x) + \sum A_n \cos [(x - L)k_n] \exp [-(k_n^2 + 1)t]$$

where $t$ is time normalized to the membrane time constant $\tau$, $k_n$ is eigen values, and $A_n$ is coefficients. For the case of
field switch-off, the stationary component $W''(x) = 0$ while the transient component changes sign. Individual components of the transient decay with a corresponding time constant, $\tau_n = T/(k_n^2 + 1)$. The eigen values, $k_n$, obey the transcendental equation

$$k_n \tan (L \cdot k_n) = \omega/S$$

which is analogous to that in the case of current injection through a microelectrode (Durand 1984; Kawato 1984; Rall 1969). The coefficients can be expressed in the following way (APPENDIX B)

$$A_n = 2E[\cos (Lk_n) - 1] / \left[ L + \omega \cos^2 (Lk_n) \right] (k_n^2 + 1)$$

A sample distribution of the transmembrane potential is presented in Fig. 1A.

If the shunt is absent, $S = \infty$, the transient after current injection may be divided in two parts (Rall 1969, 1989). The fast component is due to charge equalization along the injection may be divided in two parts (Rall 1969, 1989). The coefficients can be expressed in the following way (APPENDIX B)

$$A_n = 2E[\cos (Lk_n) - 1] / \left[ L + \omega \cos^2 (Lk_n) \right] (k_n^2 + 1)$$

A sample distribution of the transmembrane potential is presented in Fig. 1A.

For further qualitative analysis, we computed the effect of a field step on the transmembrane potential in a three-dimensional reconstructed hippocampal interneuron (Thurbon et al. 1994) and a turtle motorneuron (Fig. 2C in Ruigrok et al. 1984). These two neurons were chosen because of their different branching pattern: the hippocampal interneuron is very asymmetric and has one long apical dendrite and several short basal dendrites (Fig. 1C, inset), whereas the motorneuron has almost symmetrical dendritic branching (Fig. 2A). We do not consider the reliability of the parameters used because the basic phenomena are observed in a wide range of parameters.

For the three-dimensional reconstructed hippocampal interneuron, we have used the parameter values suggested for the neuron considered (Thurbon et al. 1994): $R = 14.4$ kΩ·cm$^2$, $\rho = 410$ Ω·cm, and $C = 1$ nF/cm$^2$. Hence, for a dendrite of 1 μm in diameter, length constant $\lambda = 296$ μm and characteristic resistance $\omega = 1.547$ GΩ. For a dendrite with a diameter, $D$, $\lambda = \lambda D^{1/2}$, and $\omega = \omega D^{3/2}$. In the communication mentioned above, an apparent $R_m = 102$ MΩ is reported for whole cell recording and one-fifth of the input conductance is attributed to a shunt. The “true” $R_m = 125$ MΩ in whole cell recording is 2.5- to 5-fold larger than observed in intracellular recording (Lacaille et al. 1987). For the shunt $S = 59$ MΩ, the resulting apparent input resistance is 40 MΩ, which coincides with the value obtained with sharp electrodes (Lacaille et al. 1987). With such a shunt, over- and undershoots attain almost one half of the steady state polarization level (Fig. 1C). Over- and undershoots disappear when the $S$ is >10-fold larger than the “true” $R_m$ (not shown). It is of interest to examine the relationship between the relative magnitude of the over- and undershoots and the electrotonic parameters of the reconstructed neuron. After fixing the apparent $R_m = 30$, 40, or 50 MΩ by choosing an appropriate $S$, we have varied the parameter $\lambda$ values for a branch of 1 μm in diameter and computed $\omega$ from $\lambda$ and $R_m$. The relative value of the overshoot and undershoot grows with increasing $\lambda$ (Fig. 1C). It becomes immeasurably small only at $\lambda$ values lower than the range $\lambda = 0.2$–2 mm now considered possible (Major et al. 1994; Rapp et al. 1994; Thurbon et al. 1994).
The reconstructed apical dendrite is much longer and thicker than the basal dendrites (Thurbon et al. 1994). The neuron is devoid of large laterally oriented dendrites, which shunt field-induced somatic polarization (not the charge, however). These circumstances facilitate the manifestation of the field induced polarization.

To compare theoretical and experimental results, we also computed the field effect for a quasi-reconstructed turtle motor neuron (Ruigrok et al. 1984). This neuron has approximately symmetrically branching dendrites (Fig. 2A). The chosen electrotonic parameters were: $\tau = 20$ ms, $\lambda = 410$ $\mu$m, $\omega = 1,560$ M$\Omega$, where $\lambda$ and $\omega$ are calculated for a cable with a diameter of 1 $\mu$m. Using these parameters, the input resistance of the model neuron and the characteristic time of the potential decay after the field step fell in the range of values observed experimentally (see next section). The calculated transients of the transmembrane potential (Fig. 2B) qualitatively match that for the cable and the hippocampal interneuron.

Although over- and undershoots were good indicators of a shunt in the examples above, they also were observed in some special cases in the absence of a shunt (not shown). For the motoneuron considered, it was possible to induce a nonmonotonic response in a narrow range of field directions associated with low steady state polarization. In all such cases, in accordance with the theoretical results presented, the over- and undershoots decayed with a time constant much smaller than the membrane time constant. Therefore, it is necessary to measure both the field- and current-induced responses to detect the presence of a shunt.

As the effect of the field depends on the field strength, the electrotonic structure, and the symmetry of dendritic branching, it is difficult to define the lowest value of the shunt that can be detected. An indication is provided by the following estimate, however. The cable-like dendrite represents the extreme case of branching with the largest response to field stimulation. If the cable length is $l$, the noise level is <20 $\mu$V, and field strength is $1$ mV/$l$, it is possible to detect a shunt with a resistance as high as $10 R_m$ (Fig. 1B). By increasing field strength, a smaller shunt can be detected, but this increases the possibility of activating potential dependent currents.

Experiments

Voltage-sensitive conductances in dendrites and cell bodies can be activated by applied electric fields (Chan et al. 1988; Hounsgaard and Kiehn 1993; Lopez et al. 1991). For this reason, the field stimulation was chosen to be weak: an applied field current of 0.1 mA induced an extracellular potential difference of 4 mV from the central canal to the lateral edge of the ventral horn. The response to field stimulation is largest in the electrically compact cell (Gutman and Svirskis 1995) in which the intracellular potential is the same everywhere. The maximal transmembrane potential induced is therefore, at most, 2 mV, which we assume not affect active conductances.

Intracellular recordings

Motoneurons (42) and interneurons (6) were recorded with sharp electrodes. The input resistance ranged from 10 to 50 M$\Omega$ for motoneurons and from 64 to 180 M$\Omega$ for interneurons. These ranges are similar to those obtained in earlier studies (Hounsgaard and Kjaerulf 1992; Hounsgaard et al. 1988). The resting membrane potential recorded in motoneurons was $-72 \pm 7$ mV and spike height was $98 \pm 8$ mV, in interneurons they were $-61 \pm 5$ and $92 \pm 12$ mV, respectively.

Cells (18) were checked for over- and undershoots. In seven cases, the response was nonmonotonic (Fig. 2C) and decayed with the same slowest time constant as the response to a current pulse injected through the recording electrode (Fig. 2C, inset). The responses to the field and current stimulus of opposite polarity had the same properties (not shown). This suggested the presence of an injury shunt. In the remaining cases, there were no noticeable over- or undershoots (Fig. 2D).

In a few cells with nonmonotonic responses, we noted that the polarity of overshoot was different from the polarity of the steady state potential of the field response. This phenomenon is due to movement of the indifference point during the transient field response (Fig. 1A). In this case, the indifference point should pass through the recording site. The proximity of the recording site to the indifference point is supported by the low steady state level of the field response.

Whole cell recordings

Records were obtained from 12 motoneurons and 24 interneurons with patch electrodes. Input resistance ranged from 12 to 40 M$\Omega$ for motoneurons, and from 50 to 500 M$\Omega$ for interneurons. The resting membrane potential was $-58 \pm 7$ mV and spike height was $106 \pm 9$ mV for motoneurons and $-58 \pm 5$ mV, $97 \pm 12$ mV for interneurons, respectively.

In this recording mode, we first checked whether the seal quality was changed by the rupture of the membrane. A spike was induced by a strong field before and after the rupture of the membrane. In the cell-attached voltage-clamp mode, we determined the minimal field current for spike induction (Fig. 3A). The same procedure was repeated in current-clamp mode just after the rupture of the membrane. A change in threshold field needed to elicit a spike was taken to indicate a change in seal resistance. This conclusion was confirmed by the observation ($n = 3$) that a reduced threshold was associated with the presence of slowly decaying over- and undershoots in the weak field-induced responses (Fig. 3D). In seven motoneurons and six interneurons, there was no change in the threshold field. In these neurons, responses to the weak field stimulation were monotonic and fast. The field-induced transient decayed about five times faster than the response to a current pulse. This means that the seal was perfect, the soma-dendritic membrane had uniform passive properties in the voltage range tested, and the influence of the axon was small.

To check theoretical inferences, we induced an inhomogeneity by applying glycine near the recording electrode ($n = 4$). During the application of glycine the input resistance decreased (Fig. 3C), the response to the current pulse became faster (Fig. 3B) and slowly decaying over- and undershoots appeared (Fig. 3B).
We have presented a theoretical analysis of the impact of a local shunt on the polarization induced by an electric field. The analysis predicted that in neurons with homogeneous passive membrane properties the transient response to DC field polarization should decay faster than the response to a current pulse injected through the recording electrode. In a shunted neuron, there should be field-induced slow over- and undershoots decaying with the same slow rate as the response to injected current. According to Eq. 7, the shunt may be nonohmic, because nonlinear inhomogeneities also cause a non-zero total induced charge. The theoretical predictions were checked experimentally using sharp and patch electrodes.

In the present study, the term shunt may include three components: a direct electrical contact between the cell’s interior and exterior, i.e., a “hole”; a decrease in proximal membrane resistance due to injury-mediated changes in composition of the cytoplasm, for example, due to a change in Ca\(^{2+}\) concentration (Clements and Redman 1989; Staley et al. 1992); and changed activation of ion channels due to a shift in membrane potential caused by the first two components.

It follows from Eq. 7 that slow over- and undershoots may occur due to an injury shunt or a marked inhomogeneity in the passive properties of the membrane polarized by the field. A practically important case of an inhomogeneity is polarization of the axon (Gutman and Svirskis 1995; Tranchina and Nicholson 1986). The absence of slow over- and undershoots may be used to verify the common assumption that neurons have homogeneous ohmic membranes when active conductances are not recruited.

In our case, the experimental results obtained with patch electrodes suggest that the soma-dendritic membrane of ventral horn cells in the turtle spinal cord is homogeneous in the voltage range where active conductances are not recruited and that the effect of the axon is insignificant.

It was found that sharp electrodes in some cases induce under- and overshoots in response to the field stimulus, suggesting the presence of an injury shunt in these cases. Nevertheless, a majority of cells impaled with sharp electrodes had no detectable shunt.

The average membrane potential recorded with sharp electrodes was about \(-70\) mV in motoneurons, which is 10 mV more hyperpolarized than obtained with patch electrodes. This difference is most likely due to the junction potential of the patch electrode. In agreement, spike generation required a similar threshold depolarization from the resting membrane potential in the two recording modes. Activation of a calcium-dependent potassium conductance and/or pump in recordings with sharp electrodes evoked by calcium influx through a membrane shunt or by damage to internal calcium sequestering is another conceivable source of difference in membrane potential. However, the absence of a detectable shunt in most recordings and the similar input resistance obtained with the two recording modes does not support this explanation.

It should be noted, that in the present study we used sharp electrodes pulled from thick-walled glass tubes to get a lower capacitance for voltage-clamp experiments. This may explain some discrepancy with the earlier studies done with finer microelectrodes (Hounsgaard et al. 1988), where the resting membrane potential in motoneurons was about \(-60\) mV rather than \(-70\) mV in present study. However, the range of input resistances is the same in the two studies.

There were only minor differences in firing mode and passive properties in recordings with sharp and patch electrodes. Possibly, the relatively high access resistance (>15 M\(\Omega\)) in the whole cell recordings prevented wash-out of intracellular medium. The only difference was that with the patch electrodes it was possible to record from cells with much higher input resistances, possibly smaller cells. Maybe, only larger cells survive penetration with the sharp electrodes (see also Spruston et al. 1994; Staley et al. 1992); survival, however, does not mean absence of injury. Although our data base did not allow a detailed statistical analysis, we did

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**FIG. 3.** A: responses to a strong DC field stimulus before (top, voltage clamp) and after (bottom, current clamp) rupture of membrane. Stimulus in a did not induce a spike in either case whereas slightly larger stimulus in b was suprathreshold in both cases, suggesting that the seal was not affected by membrane rupture. B: responses to weak field and current pulse stimulation in same neuron. Response to field was monotonie and fast (grey line), indicating absence of a shunt. Glycine application near recording electrode induced under- and overshoots (black line) and response to current pulse became faster, reflecting presence of a shunt. C: responses to a long current pulse before (-----) and during application of glycine (----), note larger than twofold decrease in input resistance. D: nonmonotonic response to weak field stimulation in a patch recording. In this case, threshold field for spike induction became lower after rupture of the membrane. A–C from same cell. f, field current and c, microelectrode current.
not notice a correlation between observed overshoots and the values of resting membrane potential, spike amplitude or input resistance. This indicates that overshoots are sensitive indicators of injury and membrane inhomogeneity.

In the set of cells studied here, the input resistance obtained with sharp electrodes is similar to the input resistance with whole cell measurements and significantly higher than in similar sized cells in slice preparations from adult mammals. This probably reflects the as yet unexplained favorable recording conditions in isolated preparations from the turtle (Hounsgaard and Nicholson 1990). The large difference in input resistance obtained with the two recording modes in the hippocampus and the neocortex of neonatal rats should be reflected in the field responses of the neurons.

The detection of an injury shunt may be particularly useful in studies aimed at evaluating electrotonic parameters because it would decrease the number of free parameters and make the estimates more robust. It is significant to note that the small polarization needed for shunt detection (<2 mV) makes the technique useful even in neurons, which, like motoneurons (Hounsgaard and Kiehn 1993; Larkum et al. 1996), have nonlinear membrane properties in cell bodies and dendrites. The field-induced polarization itself can be used for estimating the electrotonic structure (Gutman and Svirskis 1995) and for checking the standard methods of electrotonic measurements (Holmes and Rall 1992b; Major et al. 1994; Thurbon et al. 1994).

The major effects predicted and evaluated here are qualitative rather than quantitative. The qualitative implications do not require cell reconstruction and field homogeneity. This suggests that these methods could be used for shunt detection in other cells and other preparations in vitro and in vivo.

**APPENDIX A**

Let us consider the change in transmembrane potential induced by a homogeneous DC field in a homogeneous cable. We take into account only the field-induced changes of the transmembrane potential that are superimposed on the shunt-induced initial potential distribution.

Because the extracellular potential $U = -Ex$, the cable equation for the transmembrane potential, $W = V - U$, is

$$\frac{\partial W}{\partial x^2} - \frac{\partial W}{\partial t} = W \quad (A1)$$

where $x$ and $t$ are in electrotonic units. When a shunt, $S$, is present at the proximal end of the cable and the distal end is sealed, the boundary conditions for the intracable potential, $V$, are

$$\left.\frac{\partial V}{\partial x}\right|_{x=0} = \frac{\omega}{S}W \quad \text{and} \quad \left.\frac{\partial V}{\partial x}\right|_{x=L} = 0 \quad (A2)$$

For the transmembrane potential we get

$$\left.\frac{\partial W}{\partial x}\right|_{x=0} = E + \frac{\omega}{S}W \quad \text{and} \quad \left.\frac{\partial W}{\partial x}\right|_{x=L} = E \quad (A3)$$

The boundary conditions, Eq. $A3$, are formally equivalent to current injection at both ends of the cable. After a long-lasting DC field step, we have the stationary solution. Its general form is as follows: $W(x) = A\sinh(x) + B\cosh(x)$. Substituting this expression into the boundary conditions we can find the unknown coefficients

$$A = E[\omega/S + \sinh(L)]/\{\sinh(L) + \omega/S \cdot \cosh(L)\} \quad (A4)$$

$$B = E[1 - \cosh(L)]/\{\sinh(L) + \omega/S \cosh(L)\} \quad (A5)$$

Finally, the stationary solution for the transmembrane potential distribution is the following

$$W(x) = E \left( \cosh(x) + \frac{\omega}{S} \sinh(x) - \cosh(x - L) \right) / \left( \sinh(L) + \frac{\omega}{S} \cosh(L) \right) \quad (A6)$$

**APPENDIX B**

The general solution of the Eq. $A1$ usually is expressed in the form $W(x,t) = W(x) + T(x,t)$, where $W(x)$ is the stationary part and $T(x,t)$ is the transient part (Durand 1984). The transient part has to satisfy the boundary conditions (Eq. $A3$) when $E = 0$, because the DC field effect already is taken into account by the stationary part. So the problem for the transient is the same as in the case of current injection. The expression for the transient part of the solution is easily found employing the method of variable separation and Fourier expansion (Durand 1984; Rall 1969, 1989)

$$T(x,t) = \sum_n A_n \cos \left[ \left( x - L \right) k_n \right] \exp \left[ -(k_n^2 + 1)t \right] \quad (B1)$$

where eigen functions were chosen to satisfy the boundary condition at the distal end. The eigen values, $k_n$, obey the transcendental equation, which follows from the boundary conditions at the proximal end

$$k_n \cdot \tan (L \cdot k_n) = \omega / S \quad (B2)$$

The coefficients $A_n$ are calculated using the initial condition, $W(x, 0)$

$$A_n = \int_0^L \left[ W(x, 0) - W(x) \right] \cos \left[ \left( x - L \right) k_n \right] dx / \int_0^L \cos^2 \left[ \left( x - L \right) k_n \right] dx \quad (B3)$$

In the case of the onset of the field step, the initial condition $W(x, 0) = 0$. The stationary distribution of the transmembrane potential, $W(x)$, is Eq. $A6$. In the case of switching off the long lasting field step, the initial condition $W(x, 0)$ is equal to Eq. $A6$ and the stationary distribution $W(x) = 0$. So the coefficients in both cases differ only in sign. For the first case we get by integration

$$A_n = 2E \left[ \cos (Lk_n) - 1 \right] / \left( L + \omega \cos^2(Lk_n) \right) (k_n^2 + 1) \quad (B4)$$

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REFERENCES


BADGLEY, J. B. Cable analysis with the whole-cell patch clamp. Theory


