Comparative Electrotone Analysis of Three Classes of Rat Hippocampal Neurons

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Carnevale, Nicholas T., Kenneth Y. Tsai, Brenda J. Claiborne, and Thomas H. Brown. Comparative electrotone analysis of three classes of rat hippocampal neurons. J. Neurophysiol. 78: 703–720, 1997. We present a comparative analysis of electrotone in the three classes of principal neurons in rat hippocampus: pyramidal cells of the CA1 and CA3c fields of the hippocampus proper, and granule cells of the dentate gyrus. This analysis used the electrotone transform, which combines anatomic and biophysical data to map neuronal anatomy into electrotone space, where physical distance between points is replaced by the logarithm of voltage attenuation (log A). The transforms were rendered as “neuromorphic figures” by redrawing the cell with branch lengths proportional to log A along each branch. We also used plots of log A versus anatomic distance from the soma; these reveal features that are otherwise less apparent and facilitate comparisons between dendritic fields of different cells. Transforms were always larger for voltage spreading toward the soma (Vout) than away from it (Vin). Most of the electrotone length in Vout transforms was along proximal large diameter branches where signal loss for somatofugal voltage spread is greatest. In Vin transforms, more of the length was in thin distal branches, indicating a steep voltage gradient for signals propagating toward the soma. All transforms lengthened substantially with increasing frequency. CA1 neurons were electrotone significantly larger than CA3c neurons. Their Vout transforms displayed one primary apical dendrite, which bifurcated in some cases, whereas CA3c cell transforms exhibited multiple apical branches. In both cell classes, basilar dendrite Vout transforms were small, indicating that somatic potentials reached their distal ends with little attenuation. However, for somatotopical voltage spread, attenuation along the basilar and apical dendrites was comparable, so the Vout transforms of these dendritic fields were nearly equal in extent. Granule cells were physically and electrotone most compact. Their Vout transforms at 0 Hz were very small, indicating near isopotentiality at DC and low frequencies. These transforms resembled those of the basilar dendrites of CA1 and CA3c pyramidal cells. This raises the possibility of similar functional or computational roles for these dendritic fields. Interpreting the anatomic distribution of thorny excrescences on CA3 pyramidal neurons with this approach indicates that synaptic currents generated by some mossy fiber inputs may be recorded accurately by a somatic patch clamp, providing that strict criteria on their time course are satisfied. Similar accuracy may not be achievable in somatic recordings of Schaffer collateral synapses onto CA1 pyramidal cells in light of the anatomic and biophysical properties of these neurons and the spatial distribution of synapses.

INTRODUCTION

By governing the spread of electrical signals, electrotone structure establishes the context for information processing in neurons. It conditions the global integration of synaptic inputs to drive spiking (Bekkers and Stevens 1990; Claiborne et al. 1992; Edwards et al. 1994; Holmes and Rall 1992; Jack et al. 1983; Rall 1977), it sets the extent of local interactions between synaptic inputs (Mainen et al. 1996; Shepherd and Koch 1990; Shepherd et al. 1989), and it is relevant to the voltage-dependent synaptic modifications that are thought to underlie certain types of learning (Brown et al. 1988, 1990–1992; Fisher et al. 1993; Kairiss et al. 1992; Kelso et al. 1986; Mainen et al. 1990, 1991; Tsai et al. 1994a). Any understanding of the consequences of active currents for neuronal function must take electrotone structure into account, because it provides the framework within which the signals generated by active currents spread and interact (Gillessen and Alzheimert 1997; Jaffe et al. 1992; Lipowsky et al. 1996; Magee and Johnston 1995; Schwindt and Crill 1995; Stuart and Sakmann 1995); recent modeling (Mainen and Sejnowski 1966) suggests that electrotone structure may be a critical determinant of neuronal firing patterns. Furthermore, electrotone structure is important to the design and interpretation of experimental studies of synaptically mediated and voltage-gated currents and potentials (Barrionuevo et al. 1986; Carnevale et al. 1994; Cauller and Connors 1992; Claiborne et al. 1993; Jaffe and Brown 1994; Jaffe and Johnston 1990; Jaffe et al. 1994; Johnston and Brown 1983; Johnston et al. 1992; Kairiss et al. 1992; Mainen et al. 1996; Siegel et al. 1992; Spruston et al. 1993, 1994).

Although mammalian neurons can be classified on the basis of morphological differences, understanding the relevance of these differences to electrotone requires that anatomy be interpreted in the context of biophysics. To this end, we have explored various new approaches to the problem of defining and analyzing the consequences of cellular anatomy and biophysics for electrical signaling in neurons (Brown et al. 1992; Carnevale et al. 1995a,b; O’Boyle et al. 1993, 1996; Tsai et al. 1993, 1994b; Zador et al. 1995). We have developed a method that combines these properties, mapping the branched architecture of a neuron into “electrotone space” through a transformation that lends itself to graphic displays that provide a quick and intuitive grasp of the spread of current and voltage (Carnevale et al. 1995a; O’Boyle et al. 1996; Tsai et al. 1993, 1994b).

We implemented the transformation with a powerful, ef-
icient algorithm that makes it practical to study a large number of cells with unprecedented resolution in frequency and space. Using this approach, we have analyzed electrical signaling in the dendritic trees of three classes of rat hippocampal neurons: CA1 pyramidal neurons, CA3c pyramidal neurons, and granule cells of the dentate gyrus. This comparative analysis disclosed striking contrasts and unexpected similarities between these cells that may have important implications for hippocampal operation. These findings also suggest new strategies for neuronal classification. By virtue of its close relationship to function, the electrotonic transformation may reveal useful insights to the organization of the brain that would remain undetected by methods based solely on morphological criteria.

METHODS

Anatomic reconstructions

Adult Sprague–Dawley rats were anesthetized deeply with pentobarbital sodium (Nembutal; 60 mg/kg body wt) and decapitated. The brain was removed quickly, and 400 μm-thick slices of the middle third of the hippocampal formation were prepared and maintained at 32°C in a recording chamber (Claiborne et al. 1986). Horseradish peroxidase (HRP) was injected into pyramidal neurons of the CA1 and CA3c fields of the hippocampus and granule cells of the dentate gyrus using previously described techniques (Claiborne 1992; Claiborne et al. 1986, 1990; Rihn and Claiborne 1990; Seay-Lowe and Claiborne 1992). Cells were impaled with sharp electrodes filled with 2–3% HRP in KCl/tris (hydroxymethyl)aminomethane buffer (pH 7.6). To decrease the chance of labeling neurons whose dendrites had been severed during the slicing process, only cells located in the middle of the slice were impaled. Neurons with a resting potential of at least −60 mV were injected with HRP using 3–5 nA positive current pulses with a 250-ms duration at a rate of 2 Hz for 20–25 min.

The slices were left intact during tissue processing. After an interval of 2–3 h for HRP diffusion, they were fixed and processed with diaminobenzidine for visualization (Claiborne et al. 1990). To minimize shrinkage, they were cleared in ascending concentrations of glycerol and mounted on slides in 100% glycerol. Slices prepared in this manner shrink by <5% in linear dimension, and the dendrites are not distorted (Claiborne 1992). Therefore no corrections were needed for shrinkage or "wiggle".

HRP was the label of choice for two reasons. First, it has been shown to fill hippocampal neurons in their entirety, including the finest dendritic branches (Claiborne 1992; Claiborne et al. 1990; Ishizuka et al. 1995). Second, the histochemical process required to visualize HRP can be done with intact thick slices so there is no need for resectioning. Thus the anatomic structure of an entire neuron can be analyzed directly from a slice whole-mount. Thinner slices are required for the histochemical reactions used to visualize biocytin (reaction with avidin coupled to HRP) or to produce a dense product from the fluorescent dye Lucifer yellow (reaction with antibodies coupled to HRP).

This study included seven pyramidal cells from the CA1 field, four pyramidal cells from the CA3c field, and six granule cells from the dentate gyrus. These cells were selected because they were well filled with HRP and had a minimum number of cut branches. Staining was uniformly dense throughout, with no fading toward the dendritic tip. The numbers of cut branches in each dendritic field of the pyramidal neurons were CA1: apical, 0–6 (average 3); basilar, 0–4 (average 1.8); CA3c: apical, 0–4 (average 1.6); basilar, 1 each. None of these cuts affected a proximal or "primary" branch. Granule cells were rejected if a dendrite in the proximal half of the molecular layer was cut or if two or more branches were severed in the distal two-thirds of the layer. Further confirmation of the anatomic integrity of these cells was provided by comparing their total dendritic lengths with values that have been reported previously for granule (Claiborne et al. 1990; Rihn and Claiborne 1990) and pyramidal (Ishizuka et al. 1995) neurons of rat hippocampus in studies using the same techniques; in all cases these lengths were well within the corresponding range.

The CA3 neurons we examined were from the CA3c field, the portion of CA3 that lies closest to the hilus of the dentate gyrus. Part of the mossy fiber projection from the dentate runs through the basilar region of this field, and the transition from CA3c to CA3b is approximately at the distal end of this infrapyramidal bundle (Lorente de Nó 1934). The morphology of pyramidal cells in CA3c is reportedly more heterogeneous than in CA3a or CA3b (Scharfman 1993). We selected CA3c because these cells are frequently the target of physiological investigations. They are of particular interest to the study of synaptic transmission in mammalian brain (Xiang et al. 1994) because their relative proximity to the dentate may favor the experimental isolation of a pure, monosynaptic mossy fiber input (Claiborne et al. 1993).

Camera lucida drawings of filled neurons were made using a ×63 objective (Zeiss Neofluar oil immersion, working distance 0.5 mm, NA 1.25) attached to a Nikon Optiphot microscope. Three-dimensional reconstructions of cells were obtained directly from the thick slices using a computer-microscope system designed by John Miller (University of California, Berkeley), with software written by Rocky H.W. Nevin (Claiborne 1992; Jacobs and Nevin 1991; Nevin 1989; Rihn and Claiborne 1990). The system consisted of a Nikon Optiphot microscope interfaced to an IBM AT computer that controlled motors mounted on both the microscope stage and the focus-control knob. Accurate positioning of the stage was ensured by optical encoders capable of 0.2 μm resolution. Labeled neurons were digitized in three dimensions by an operator using a computer mouse. A video camera was mounted on the microscope and the dendrites were viewed on a monitor. Diameter measurements were taken from a reference cursor superimposed over the dendrite on the monitor (Rihn and Claiborne 1990). Each datum included XYZ coordinates and a diameter measurement. Further details are provided elsewhere (Claiborne 1992; Claiborne et al. 1990).

The effect of dendritic spines on cell electrical properties is often compensated by adjusting surface area or membrane properties based on spine dimensions and density (Caulder and Connor 1992; Claiborne et al. 1992; Stratford et al. 1989). However, a significant variation of spine density with dendritic diameter recently has been reported in CA1 pyramidal neurons (Bannister and Larkman 1995), and significant if less striking variation than long has been recognized in granule cells (Desmond and Levy 1985). Furthermore, even within a single cell class there can be a wide range of spine dimensions (Chicurel and Harris 1992; Desmond and Levy 1985; Harris et al. 1992), so it is unclear how large this compensation should be. In addition, our laboratories recently have been exploring the use of confocal scanning laser microscopy to improve the accuracy of diameter measurements (O’Boyle et al. 1993, 1996). Diameters tend to be overestimated by as much as 0.5–1.0 μm when standard light microscopic techniques are applied to thick slices (O’Boyle et al. 1993). The resulting increase of apparent surface area amounts to ~1.6–3.1 μm²/μm length, which brackets the weighted estimate of 2.85 μm²/μm that we previously derived (Mainen et al. 1996) from measurements in CA1 pyramidal neurons reported by Harris et al. (1992). Therefore in this study, we made no alterations in membrane properties or measured diameters and thereby accomplished a partial compensation for the effect of dendritic spines. This seemed preferable to compounding the uncertainties of
Electrotonean analysis

Because of the central importance of $R_c$ and $R_m$ to the construction of the transforms, we based the values we used on the results of Spruston and Johnston (1992), who exercised great care to obtain measurements that were as physiologically and as accurate as possible. The passive electrical properties were $R_c = 200 \, \Omega \cdot cm$, $C_m = 1 \, \mu F/cm^2$ for all three cell classes, and $R_m = 30 \, k\Omega \cdot cm^2$ for CA1 pyramidal cells, 70 $k\Omega \cdot cm^2$ for CA3 pyramidal cells, and 40 $k\Omega \cdot cm^2$ for granule cells. As we have noted elsewhere (Mainen et al. 1996), these should be regarded as “linearized” rather than “passive” parameters, because the defining experiments did not employ channel blockers or attempt to inactivate currents and membrane potential changes were kept within the linear range of the cells’ current-voltage relationships (Spruston and Johnston 1992). Therefore these parameter estimates are a linearized approximation of all active and passive mechanisms that contributed to the total clamp current for potential fluctuations within $\pm 5 \, mV$ of resting potential.

Transforms were performed at several frequencies because of the frequency dependence of attenuation. For the purpose of illustration, we show the transforms at DC ($0 \, Hz$) and $40 \, Hz$.

The cells were mapped into electrotonean space by computing the attenuation of voltage for signal spread away from ($V_{i\rightarrow j}$) or toward ($V_{j\rightarrow i}$) the soma. This mapping or transformation is presented from a more theoretical standpoint elsewhere (Tsai et al. 1994b). The following sections briefly review the transformation and previously undescribed computational strategies that enable its practical application.

Signal attenuation in neurons

The principles that underlie our analytic strategy derive from the application of two-port network theory to linear electrotonean by Carnevale and Johnston (1982). Their use of two-port theory was motivated by the fact that the spread of electrical signals in a neuron is best described in terms of the efficacy of signal transfer. Three principal conclusions of their work have a major bearing on our new approach: the direction-dependence of signal transfer, the identity of current and charge transfer, and the directional reciprocity between the transfer of voltage and the transfer of current and charge.

Signal transfer is direction-dependent. Carnevale and Johnston (1982) described the loss of amplitude suffered by a signal that propagates through a neuron with a factor $k$. This factor was always $\le 1$ because it was the ratio of the “downstream” (output) amplitude to the “upstream” (input) amplitude. The electrotonean transform uses the inverse of this ratio because it leads to a natural definition of electrotonean distance.

For any two points $i$ and $j$ in a cell, if a voltage $V_i$ applied at upstream location $i$ produces a voltage $V_j$ measured at location $j$, we define the voltage attenuation to be $A_{ij} = V_j/V_i$. If the direction of propagation is reversed, so that $j$ is upstream relative to $i$, the voltage attenuation is $A_{ji} = V_i/V_j$. Because of the direction dependence of signal transfer (Carnevale and Johnston 1982), these attenuations will generally not be equal

$$A_{ij} \neq A_{ji} \quad (1)$$

The degree of inequality depends on factors such as anatomic asymmetry, regional variation of biophysical properties, and the locations of $i$ and $j$. Current and charge attenuation are also direction dependent.

Mapping from anatomic to electrotonean space

Equations 1 and 2 show that a complete description of electrotonean in a neuron requires knowledge of the attenuation of electrical signals along each branch of the cell in two directions. The identity of current and charge transfer (Eq. 3) and the directional reciprocity between voltage and current transfer (Eq. 4) imply that electrotonean could be specified equally well in terms of the attenuation of voltage or current. However, voltage attenuation is the most pragmatic choice because of the central importance of membrane potential to neuronal function.

Starting from these premises, we have shown that the electrotonean structure of a neuron is defined completely by two sets of voltage attenuations: the attenuation of voltage as it propagates away from and toward a reference location (Brown et al. 1992; Tsai et al. 1994b). We then advanced a new definition of electrotonean distance, the logarithm of attenuation, which is a metric for mapping the architecture of the cell from anatomic to electrotonean space (Brown et al. 1992; Tsai et al. 1994b).

We combine detailed, accurate morphometric data with the best available estimates of the biophysical properties of membrane and cytoplasm to calculate these attenuations at DC and several frequencies of interest along each of the branches of a cell. This accomplishes a partial mapping of physical space into electrotonean space.

The next step is to organize these attenuations around a reference location. For each point of interest in the cell, we must find the total attenuation for voltage signals propagating away from and toward the reference location. Figure 1 illustrates how this is done. The endpoints of two adjacent branches are labeled as $i$, $j$, and $k$, where $j$ is the junction between the two branches. From the anatomy and biophysics of this cell, we already have computed the attenuation

Suppose a current $I_i$ enters the cell at $i$, and a voltage clamp is attached to the cell at $j$. The current attenuation $A_{ij}$ is the ratio of $I_j$ to the current $I_i$ measured by the clamp ($A_{ij} = I_j/I_i$). If the sites of current entry and clamp attachment are exchanged, the current attenuation is $A_{ji} = I_i/I_j$. As with voltage attenuations (Eq. 1), the direction-dependence of signal transfer implies that

$$A_{ij} \neq A_{ji} \quad (2)$$

Current and charge transfer are identical. Carnevale and Johnston (1982) showed that the propagation of charge $Q$ and current are equally efficient

$$A_{ij}^Q = A_{ij}^I \quad (3)$$

Directional reciprocity of voltage and current/charge transfer. The third and final conclusion of two-port theory that we need here is the fact that voltage transfer in one direction is identical to the transfer of current and charge in the opposite direction

$$A_{ij}^V = A_{ji}^I \quad (4)$$

FIG. 1. The total attenuation over path $ik$ is the product of the attenuations along the branches $ij$ and $jk$. See text for details.
tions along these two branches for the two directions of signal flow: $A_i$ and $A_k$ (Fig. 1, left), and $A_i$ and $A_j$ (Fig. 1, right).

Suppose $i$ is the reference location. Then the total attenuation for voltage spreading from $i$ to $k$ is the product of the attenuations along each branch that lies on the direct path starting at $i$ and ending at $k$. Because there are only two branches along this path, we have

$$A_{ik} = A_i A_k$$  \hspace{1cm} (5)$$

We say that $A_{ik}$ is part of the $V_{out}$ transform with respect to reference location $i$. The product of the attenuations along this same path, but in the opposite direction, gives the total voltage attenuation from $k$ to $i$

$$A_{ji} = A_j A_i$$  \hspace{1cm} (6)$$

where $A_{ji}$ is part of the $V_{in}$ transform with respect to $i$.

The extension to cases where the direct path from the location of interest $k$ to the reference location $i$ involves more than two branches is straightforward. For voltage propagating in one direction along path $ik$, the total attenuation equals the product of the attenuations of voltage propagating along the intervening branches in the same direction.

To summarize, the transformation from anatomical to electrotonic space is started by computing the attenuations in both directions along each branch of the cell and completed by multiplying these attenuations in proper combination and order so as to find the total attenuation between the reference location and each point of interest in the cell. Any point in the cell could be used as the reference, but the soma is generally a good choice. With a somatic reference, the $V_{out}$ transform reveals the influence of somatic potentials on voltage-dependent mechanisms of synaptic plasticity in the dendrites, and the $V_{in}$ transform suggests the ability of dendritic synaptic inputs to drive spiking at the cell body (Brown et al. 1990–1992; Fisher et al. 1993; Kairiss et al. 1992; Mainen et al. 1990, 1991; Tsai et al. 1994a,b). A nonsomatic reference may be more useful for studies of interactions among dendritic synaptic inputs (Carnevale et al. 1995a).

A NEW DEFINITION OF ELECTROTONIC DISTANCE. Long lists of numbers, such as tables of morphometric data or signal attenuations, are ill-suited for human use. A graphic representation is a better vehicle for communicating a large body of information in a way that fosters the rapid development of qualitative insights. For example, morphometric data can be rendered as two-dimensional projections that portray the anatomy of the cell, emulating the traditional microscopic images obtained by camera lucida drawings or photography. The length of each branch in such a figure is related directly to the physical distance between corresponding branch points in the cell.

The key to developing an intuitive graphic depiction of the electrotonic architecture of a neuron is to define a measure of “electrical distance” that expresses the signal attenuation between locations in the cell in a consistent manner. Then points widely separated in electrotonic space would correspond to anatomical locations that are poorly coupled to each other (large attenuations), whereas points that are adjacent would correspond to sites that are nearly isopotential (attenuations close to 1).

To this end we have advanced a new definition of electrotonic distance as the natural logarithm of voltage attenuation (Brown et al. 1992; Tsai et al. 1994b). Like attenuation, this electrotonic distance $L$ is direction dependent. That is, each pair of anatomic locations $i$ and $j$ is associated with two different electrotonic distances: $L_{ij} = \ln A_{ij}$ for signal propagation from $i$ to $j$ and $L_{ji} = \ln A_{ji}$ for the opposite direction. At every frequency of interest, each branch of the cell has two representations with different lengths in electrotonic space.

Our definition of $L$ has the special property that a cascade of attenuations translates into a sum of distances in electrotonic space. In other words, electrotonic distances are additive over a path that has a consistent direction of signal propagation. Thus if location $j$ is on the direct path between locations $i$ and $k$, as in Fig. 1, then $L_{ik} = L_{ij} + L_{jk}$ and $L_{jk} = L_{kj} + L_{ij}$. This unique property is a consequence of Eqs. 5 and 6 and the definition of $L$ as the logarithm of attenuation (Carnevale et al. 1995a; Tsai et al. 1994b). As we note in the next section, it allows $L$ to be used as the metric for graphic representations of mappings from anatomic to electrotonic space.

GRAPHIC RENDERS OF THE ELECTROTONIC TRANSFORMATION. For the electrotonic transform to be useful, its results must be presented in a form that is functionally relevant and easily understood. This requires rendering the electrotonic distances in convenient graphic forms.

The most intuitive graphic representations are the “neuromorphic figures” (Brown et al. 1992; Carnevale et al. 1995a; Tsai et al. 1993, 1994b), in which the branching pattern of the cell and the relative orientations of the branches are preserved but the physical branch lengths are replaced by segments that are proportional to their electrotonic lengths. These are generated in pairs, one image using the electrotonic lengths of the branches for voltage spread away from the reference location ($V_{out}$) and the other using the electrotonic lengths for voltage spread toward the reference location ($V_{in}$). Because attenuation also depends on frequency, we generate a pair of these graphs at each frequency of interest. Because of the directional reciprocity of voltage and current or charge attenuation, the renderings of $V_{out}$ and $I_{out}$ transforms are identical, as are the renderings of $V_{in}$ and $I_{in}$ transforms.

An alternative rendering plots the electrotonic distance $L = \ln A$ as a function of physical distance $x$ from the reference location (O’Byrne et al. 1996). This enables convenient evaluation of synaptic inputs that have a laminar organization and reveals the spatial voltage gradient along neurites clearly. As with the neuromorphic figures, these “$\log A$ versus $x$” plots are generated in pairs, one for voltage propagation away from ($V_{out}$) and the other for voltage propagation toward ($V_{in}$) the reference location.

The voltage attenuation between any two points in the cell can be found by combining the appropriate segments of the somatocentric $V_{in}$ and $V_{out}$ transforms (Tsai et al. 1994b). Regardless of what reference location $s$ we initially select for the $V_{in}$ and $V_{out}$ transforms, the additive property of $L$ makes it easy to generate the transforms for any other reference location $w$. The only difference between using $s$ or $w$ as a reference is in the direction of signal propagation in the branches along the direct path between these two points, where $V_{in}$ relative to $s$ is the same as $V_{out}$ relative to $w$ and vice versa. Changing the reference location does not affect the direction of signal flow in the remainder of the cell, so the attenuations along all other branches and their corresponding representations in electrotonic space are unaltered. The additive property of $L$ is responsible for this simple relationship. Without it, generating the transforms for a new reference location would require a laborious recalulation of all the mappings from anatomic to electrotonic space.

Our approach to electrotonic analysis differs in several important ways from the traditional equivalent cylinder method. First, our definition of electrotonic distance $L$ as the logarithm of attenuation contrasts strongly with the conventional definition of electrotonic length $X$ as the ratio of the physical distance $x$ to the space constant $\lambda$ (Jack et al. 1983; Rall 1977). The classical $X$ lacks the additive property that makes $L$ so useful for graphic representations of attenuation over a chain of dendritic branches. Furthermore, attenuation is a simple exponential function of $L$, whereas its variation with $X$ is much more complicated and depends on boundary condi-
Attenuation is an exponential function of $X$ only in the case of an infinitely long cylindrical cable with uniform biophysical properties. Finally and, perhaps most importantly, the electrotonic transform encodes both anatomic and electrophysiologic data, so it does not require collapsing the cell into an equivalent cylinder and hence does not destroy the anatomic relationships among synaptic inputs distributed throughout the dendritic tree (see Mainen et al. 1996). Like our definition of electrotonic length $L$, the transform is directly applicable to any architecture.

THE TRANSFORM ALGORITHM. In principle, voltage attenuations can be determined by computing the distribution of potential in response to an applied signal using a simulator such as NEURON (Hines 1984, 1989, 1993, 1994), and this is what we did initially. However, this approach is feasible only for the DC $V_{out}$ transform. Simulation run times for non-0 frequencies were excessively long because many cycles had to pass before the response settled: a single run to find the $V_{out}$ attenuations at 40 Hz took >20 h on a SUN Sparc 10–40 (Tsai et al. 1994b) compared with a few seconds for DC. Computing a full set of $V_{in}$ attenuations would require a separate simulation using a signal applied to each terminal dendritic branch in turn. This was out of the question because the pyramidal cells in this study have ~100 terminations each; so finding the $V_{in}$ transform at one frequency for a single cell would have taken ~2000 h (almost 3 mo).

For this reason, we developed a new program that computes the $V_{in}$ and $V_{out}$ attenuations in ~2 s per frequency of interest (Tsai et al. 1994b). This program achieves its speed by operating in the frequency domain rather than the time domain, exploiting the branched architecture of a neuron to compute attenuations by a multipass recursive strategy.

The user specifies the file that contains the morphometric data, the frequencies at which the attenuations are to be calculated, and the biophysical properties of the membrane and cytoplasm. The program then reads the morphometric data and builds a model of the cell that consists of a branched tree of cylindrical segments. The internal representation of the architecture of the cell and the anatomic and electrical properties of each segment is in the form of a doubly linked binary tree (Sobelman and Krekelberg 1985; Wirth 1976).

The fundamental operation of the program is the repeated application of Kirchhoff's laws (Kuo 1966) to the equivalent circuit of the cell. This relies on the fact that each cylindrical segment can be represented by an equivalent T circuit (Fig. 2) with transverse impedance $Z_m$ and axial impedances $Z_a$ (Carnevale and Johnston 1982).

The program performs a series of recursive passes through the binary tree. Some of these passes could be combined to maximize computational efficiency, but for the sake of clarity, we present them separately. On the first pass, the morphometric and biophysical data are used to compute $Z_m$ and $Z_a$ for each segment at the frequency of interest. The values of these impedances may be approximated by simply lumping the properties of the membrane and cytoplasm

$$Z_m = \frac{2 R_l}{\pi d^2}$$

$$Z_a = \frac{R_m}{\pi d l (1 + j \omega \tau_m)}$$

where $d$ and $l$ are the segment diameter and length, $\tau_m = R_mC_m$ is the membrane time constant, $j = \sqrt{-1}$ and $\omega$ is the frequency in radians/second. These formulas are adequate for DC and low frequencies. At frequencies where the physical length of the segment exceeds 5–10% of the AC length constant $\lambda_m = \sqrt{\frac{d \rho_m}{\pi}}$, accuracy requires using these functions which we derived from cable theory (Tsai et al. 1994b)

$$\frac{\sqrt{d R_m}}{\sqrt{1 + \omega^2 \tau_m^2}}$$

$$Z_a = r_e \frac{1 - \cosh \left( \frac{x}{\lambda} \sqrt{1 + j \omega \tau_m} \right)}{\sqrt{1 + j \omega \tau_m} \sinh \left( \frac{x}{\lambda} \sqrt{1 + j \omega \tau_m} \right)}$$

$$Z_m = r_e \frac{1}{\sqrt{1 + j \omega \tau_m} \sinh \left( \frac{x}{\lambda} \sqrt{1 + j \omega \tau_m} \right)}$$

where $r_e = 2\sqrt{R_R \rho_d / \pi d^{3/2}}$ is the DC input resistance of a semi-infinite cylindrical cable of diameter $d$, $x$ is the physical length of the branch, and $\lambda = \sqrt{d R_m / \lambda^2}$ is the DC length constant.

The next four passes are illustrated through reference to the bottom of Fig. 2. In pass 2, the somatofugal input impedance at the proximal end of each segment is calculated (i.e., looking away from the soma). If the segment is a terminal branch, this is simply $Z_{out} = Z_a + Z_m$. Otherwise, the load imposed by distal segments must be included so $Z_{out} = Z_a + Z_{load}$, where $Z_{load} = Z_m(Z_a + Z_{load})/(Z_a + Z_{load})$, and $Z_{load}$ is the parallel combination of the $Z_{out}$ impedances of the daughter branches. Pass 3 calculates the $V_{out}$ attenuation along each branch as $V_{in}/V_{out} = (1 + Z_{out}/Z_m)(1 + Z_{m}/Z_{load})$. The fourth pass is similar to the second, but it starts at the soma working outward to compute the somatopetal input impedance $Z_{in}$ at the distal end of each segment (looking toward the soma). For signals propagating in this direction, the load is the (somatopetal)
TABLE 1. Maximum anatomic and electrotonic lengths

<table>
<thead>
<tr>
<th>Dendritic Field</th>
<th>Variable</th>
<th>Frequency</th>
<th>Cell Class</th>
<th>Class Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical</td>
<td>(x_{\text{max}})</td>
<td>DC</td>
<td>CA1 (n = 7)</td>
<td>847 ± 115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 Hz</td>
<td>CA1c (n = 4)</td>
<td>647 ± 128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 Hz</td>
<td>CA3c (n = 4)</td>
<td>395 ± 39</td>
</tr>
<tr>
<td>Basilar</td>
<td>(x_{\text{max}})</td>
<td>DC</td>
<td>GC (n = 6)</td>
<td>0.9 ± 0.077</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 Hz</td>
<td></td>
<td>0.94 ± 0.26</td>
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<tr>
<td></td>
<td></td>
<td>40 Hz</td>
<td></td>
<td>2.82 ± 0.67</td>
</tr>
</tbody>
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Values are sample means ± SD. Statistical significance was assessed with \(t\)-test (See METHODS). * \(x_{\text{max}}\) measured in microns.

FIG. 3. Left: two-dimensional projection of the anatomy of a CA1 pyramidal neuron (cell 524). Right: neuromorphic renderings of the electrotonic transforms of this cell computed at DC (0 Hz) and 40 Hz for somatofugal (\(V_{\text{in}}\), top) and somatopetal (\(V_{\text{out}}\), bottom) signal flow. The primary apical dendrite dominates the \(V_{\text{in}}\) transforms, while the basilar and terminal branches appear much smaller. In the \(V_{\text{in}}\) transforms the basilar and terminal branches are the most prominent features.

Statistical analysis

Anatomic distances and electrotonic lengths are reported as sample means ± SD (Table 1). Three between-class comparisons were carried out for each measure of anatomic or electrotonic extent, testing the null hypothesis that population means were equal. We used the protected \(t\)-test (Howell 1995), which is also known as Fisher’s least significant difference (LSD) test, to avoid the increased risk of Type I errors that can occur when multiple comparisons are performed with the ordinary \(t\)-test. Before performing the protected \(t\)-test, we first calculated the overall \(F\) statistic for each measure.

RESULTS

The anatomic and electrotonic architectures of representative hippocampal neurons are shown in five pairs of figures: two pairs for each of two CA1 pyramidal cells (Figs. 3–6), one pair for a CA3c cell (Figs. 7 and 8), and two more pairs for two granule cells (Figs. 9–12). The first figure of

\[ Z_{\text{in}} \] of the parent in parallel with the (somatofugal) \(Z_{\text{out}}\) of any sibling branch. The same equation that was used in pass 3 is applied in the fifth pass to find the \(V_{\text{in}}\) attenuation.

In the final pass through the tree, the attenuations are written to the output. Afterward, a new set of attenuations is computed at the next frequency of interest.

As noted above, this program evaluates attenuations several orders of magnitude faster than is possible with time-domain simulations using software such as NEURON, GENESIS, or SPICE. Computation time for our algorithm is \(O(N)\) where \(N\) is the number of compartments in the neuron model, i.e., it scales linearly with anatomic complexity. Both run time and accuracy are independent of frequency.

The power and efficiency of our program arise from two factors. The first is our use of a recursive algorithm that exploits the branched topology of the cell. Superficially, matrix methods using an upper-diagonalization-backsubstitution scheme (Carnevale and Lebeda 1987) adapted for sparse, nearly tridiagonal systems of equations might seem conceptually different, but they are computationally equivalent. A more general approach that resorts to formal matrix inversion would be inferior because the inverse of a sparse matrix is typically highly nonsparse (Jennings 1977).

The second factor that enhances computational efficiency is our strategy for circumventing the effect of increasing frequency on the effective length constant. As we pointed out elsewhere (Tsai et al. 1994b), the length constant shortens considerably starting at frequencies near \(f_m = 1/2\pi \tau_m\). This frequency is quite low for hippocampal principal neurons (~5.3 Hz for CA1 cells, ~2.3 Hz for CA3 cells, and ~4 Hz for granule cells). Approaches that lump the properties of membrane and cytoplasm into simple RC equivalents must resort to smaller compartmental size to preserve accuracy at higher frequencies (Oran and Boris 1987). Using complex impedance functions derived from cable theory eliminates the need to reduce compartmental size as frequency increases.

We have evaluated the accuracy of this program by comparing its predicted attenuations with simulations performed with NEURON (Hines 1984, 1989, 1993, 1994) using an anatomically and biophysically realistic model of a CA1 neuron. In all cases, the results agreed within 0.02%.

AVAILABILITY OF ANALYTIC TOOLS. The neural simulation environment NEURON (Hines 1993, 1994) now includes the Electrotomic Workbench (Carnevale et al. 1996), a set of analytic tools that provide a convenient way to perform the electrotomic transformation. The Electrotomic Workbench is fast and efficient because it operates in the frequency domain. Because NEURON is freely available and runs under the three leading operating systems (UNIX and its variants, all varieties of MS Windows, and the Mac OS), the transformation is maximally accessible to interested members of the neuroscience community. It can be obtained via the WWW at http://www.neuron.yale.edu and http://neuron.duke.edu

Statistical analysis

Anatomic distances and electrotonic lengths are reported as sample means ± SD (Table 1). Three between-class comparisons were carried out for each measure of anatomic or electrotonic extent, testing the null hypothesis that population means were equal. We used the protected \(t\)-test (Howell 1995), which is also known as Fisher’s least significant difference (LSD) test, to avoid the increased risk of Type I errors that can occur when multiple comparisons are performed with the ordinary \(t\)-test. Before performing the protected \(t\)-test, we first calculated the overall \(F\) statistic for each measure.

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Each pair presents the ‘raw’ anatomy of a cell on the left, with the neuromorphic renderings of its DC $V_{\text{out}}$ (somatofugal) and $V_{\text{in}}$ (somatopetal) transforms on the right (Figs. 3, 5, 7, 9, 11). The second of each pair shows the log $A$ versus $x$ plots of the transforms (Figs. 4, 6, 8, 10, and 12). The basilar dendrites of the pyramidal cells are plotted in Figs. 4, 6, and 8 at ‘negative’ ‘anatomic distances from the soma. The last figure (Fig. 15) compares log $A$ versus $x$ plots of a granule cell and the basilar dendrites of a CA1 pyramidal cell.

For each dendritic field of each neuron in this study, we also found the anatomic distance $x_{\text{max}}$ from the soma to the most remote dendritic termination, and computed the $L_{\text{out}}$ and $L_{\text{in}}$ along this path at DC and 40 Hz. In addition, we determined the anatomic and electrotonic distances of the termination(s) that were electrotonically most remote from the soma at DC and 40 Hz, i.e., the terminals that were associated with $L_{\text{out}}$ and/or $L_{\text{in}}$. Because CA1 and CA3 neurons have two dendritic fields (apical and basilar), whereas granule cells have only one, there were 34 dendritic fields (22 for the 11 pyramidal neurons, plus 12 for the 12 granule cells). Thus there were 34 values of $x_{\text{max}}$ (one for each dendritic field), 68 $L_{\text{out}}$ values (one for each field at 0 Hz and another for 40 Hz), and 68 $L_{\text{in}}$ values. These measures are plotted in Figs. 13 and 14, and their sample means ± SE are summarized in Table 1.

**General observations**

DC $V_{\text{out}}$ transforms of all three classes of neurons were relatively compact (top right of Figs. 3, 5, 7, 9, and 11), which indicates only slight to moderate attenuation of voltage spread away from the soma in the steady state. Most of the voltage drop occurred in proximal branches. Branches that were more distal are nearly invisible in these figures because they were almost isopotential from their origins to their distal terminations.

The $V_{\text{in}}$ transforms were considerably larger (bottom right of these figures) because voltage suffered more attenuation as it spreads toward the soma. Distal small-diameter branches accounted for a large fraction of attenuation in this direction, illustrating the general principle that electrotonic architecture is direction dependent (Carnevale and Johnston 1982).

In all branches of all cells, attenuation worsened with increasing frequency as a consequence of membrane capacitance, and the transforms of all cells grew more extensive. This effect was first noticeable at frequencies in the range of 5–10 Hz ($\tau \approx 32–16$ ms), and it was quite prominent at frequencies ≥40 Hz ($\tau \leq 4$ ms).

Within each neuron, the physical path lengths from the soma to the dendritic terminations were scattered over a range of values in all three cell classes. Electrotonic path lengths between the soma and dendritic terminations were also nonuniform in both directions. This confirms and extends our recently reported observations in CA1 pyramidal neurons (Mainen et al. 1996). The log $A$ versus $x$ plots show this particularly well (Figs. 4, 6, 8, 10, and 12). This nonuniformity was most pronounced in the apical dendritic trees of CA1 and CA3c cells, but it also appeared in their basilar dendrites and in granule cells.

Although $L$ tended to increase with physical distance from the soma, the dendritic branch termination that was anatomically most remote was also electrotonically most remote in only 28 of 68 cases. That is, the ‘‘$x_{\text{max}}$ path’’ was associated with both the greatest $L_{\text{out}}$ and $L_{\text{in}}$ less than half the time. The $x_{\text{max}}$ path had either $L_{\text{out}}^{\text{max}}$ or $L_{\text{in}}^{\text{max}}$ but not both in an additional 15 instances. In six cases, a physically shorter path had both $L_{\text{out}}^{\text{max}}$ and $L_{\text{in}}^{\text{max}}$.

Although either or both of these electrotonic measures was occasionally >30% larger than the corresponding $L$ of the $x_{\text{max}}$ path, the difference was usually ≤10%. Out of a total of 136 individual values of $L_{\text{out}}^{\text{max}}$ and $L_{\text{in}}^{\text{max}}$ computed at
0 and 40 Hz in these three cell classes, the largest was 10–20% greater than the corresponding \( L \) of the \( x_{\text{max}} \) path in 17 comparisons and larger yet in 14 cases.

The anatomic lengths of the \( L_{\text{out}}^{\text{max}} \) and \( L_{\text{in}}^{\text{max}} \) paths were usually no less than 90% of the \( x_{\text{max}} \) path. They were 10–20% shorter than the corresponding \( x_{\text{max}} \) in just 13 of 136 comparisons, and only two were >20% shorter. Even so, some disparities between the paths with greatest \( L \) and those with \( x_{\text{max}} \) were rather striking: in one CA3c cell a basilar dendrite was anatomically 20% shorter than the basilar \( x_{\text{max}} \) path, yet it had an \( L_{\text{out}} \) that was 19% greater at 40 Hz. At the same frequency, another CA3c cell had a basilar dendrite that was 4.4% shorter than the \( x_{\text{max}} \) path, whereas its \( L_{\text{out}} \) was 70% greater.

CA1 and CA3c pyramidal cells

The apical dendrites were the major component of the \( V_{\text{out}} \) transforms of the pyramidal cells (Figs. 3, 5, and 7). The apical dendrites were preserved through the physiologically interesting range of frequencies (0–10 kHz).

The CA1 pyramidal cells generally had a single primary apical dendrite (Fig. 3, cell 524), but in some neurons this dendrite bifurcated (Fig. 5, cell 503). It was the most prominent feature of the \( V_{\text{out}} \) transforms of CA1 cells (top right in Figs. 3 and 5), whereas its side branches were nearly invisible. This indicates that most of the attenuation for voltage propagation away from the soma occurred along its length. This is particularly clear in the log \( A \) versus \( x \) plots for \( V_{\text{out}} \) (Figs. 4 and 6), which contrast the steep longitudinal voltage gradient in the primary apicals (the long diagonal rows of points) with the nearly flat spatial profile of voltage in the side branches (the almost horizontal rows of points).

The apical dendritic trees of the CA3c pyramidal neurons were not organized around a primary stem. Instead they consisted of multiple proximal branches of similar electrotonic extent (top right of Fig. 7, cell 701) which accounted for much of the attenuation. This is especially clear in the log \( A \) versus \( x \) plots (Fig. 8), which disclose no single or bifurcating primary apical dendrite.

In the \( V_{\text{out}} \) transforms of CA1 and CA3c pyramidal cells, the basilar dendrites were very short for DC and frequencies lower than \( \omega_m = 1/\tau_m \) (top right in Figs. 3, 5, and 7). This indicates that they were virtually isopotential with the soma at low frequencies. For the particular CA1 pyramidal neuron of Fig. 5 (cell 503), the \( V_{\text{out}} \) transforms of the basilar dendrites seem to be grouped into two different electrotonic paths, but this was not a consistent feature of pyramidal cell basilar dendrites.

The relative extent of the basilar dendrites was substantially larger in the \( V_{\text{in}} \) transforms (bottom right in Figs. 3, 5, and 7). This means that voltage attenuation toward the soma along the basilar dendrites was roughly comparable with attenuation in the anatomically longer apical dendrites. This is due to the loading effect of downstream membrane on these narrow processes. The proximal end of each basilar dendrite is attached to a low impedance load: the soma and all the other dendrites that arise from it. If a synaptic input on a basilar dendrite is to evoke a voltage transient at the soma, it not only has to supply current to the membrane capacitance and conductance of the soma, but it also must supply current to the proximal ends of the remainder of the dendritic tree. Therefore producing a small potential change at the soma requires a large axial current in the basilar dendrite, which results in a steep longitudinal voltage gradient.

Granule cells

Granule cells of the dentate gyrus have a simpler branching pattern than pyramidal cells, and most dendritic terminations appear to be physically nearly equidistant from
the cell body. However, the electrotonic path lengths of the dendrites were surprisingly nonuniform. This nonuniformity could affect either the $V_{in}$ or the $V_{out}$ transform.

We present two examples of granule cells. The first shows the usual pattern of multiple branches arising close to the soma (Fig. 9, cell 964). Log $A$ versus $x$ plots at DC for the $V_{out}$ and $V_{in}$ transforms for this cell are shown in Fig. 10. The soma of the second neuron lay relatively deep in the granule cell layer (Fig. 11, cell 950) and gave rise to a single unbranched process that traveled ~30 μm before its first division.

Both cells were nearly isopotential for DC voltage spread from the soma (top right of Figs. 9 and 11). At higher frequencies, unexpected differences in attenuation emerged along their various dendritic branches (e.g., at 40 Hz in Fig. 9).

As in the pyramidal cells, there was greater attenuation of voltage propagating toward the cell body. The electrotonic path lengths from the dendritic terminations to the soma were nearly identical in some cells (Fig. 9) but nonuniform in others (Fig. 11).

The neuron with an initial solitary apical branch (cell 950) showed the loading effects of downstream membrane quite clearly. The short proximal apical branch occupied only a small fraction of the total anatomic length of dendritic tree (Fig. 11). However, voltage drop along it accounted for about one-third of the extent of the $V_{out}$ transform of this cell (top right in Fig. 11), and it was prominent in the corresponding log $A$ versus $x$ profile (Fig. 12). This is because almost the entire dendritic tree of this cell arose from its distal end, i.e., it had a very low impedance load. In contrast, the load for voltage spread in the opposite direction along this branch was quite small (just the soma). Consequently there was little signal loss, and the branch was nearly undetectable in the $V_{in}$ transforms. The difference between the $L_{out}$ and $L_{in}$ of this branch was due to the difference in the loads attached to its proximal and distal ends.

**Comparisons between classes**

Although CA1 and CA3c pyramidal neurons bear some overall resemblance to each other, there are important anatomic and electrotonic differences and similarities between these two cell classes. A rough indication of these differences and similarities is provided by comparison of the maximum anatomic ($x^{max}$) and electrotonic lengths ($L^{max}$) and Table 1, Figs. 13 and 14). The table of CA1 cells was anodically and electrotonically longer than that of CA3c cells (Fig. 13). The basilar dendrites of these two cell classes were anatomically comparable (Fig. 14A), but at DC and low frequencies, they were electrotonically more extensive in CA1 cells (Fig. 14, B and D). This difference disappeared with increasing frequency (Fig. 14, C and E).

Figure 13 also compares granule cell dendrites with the apical dendrites of CA1 and CA3c pyramidal neurons. Granule cells were anatomically significantly shorter than both classes of pyramidal neurons. They were electrotonically far more compact than the apical dendrites of CA1 cells at DC and 40 Hz. Their $L_{out}$ was comparable with CA3c apical dendrites at DC and 40 Hz, but their $L_{in}^{max}$ was significantly shorter.

We noticed certain parallels between the electrotonic transforms of granule cells and those of the basilar dendrites of CA1 and CA3c pyramidal cells. The range of attenuations and their variation with distance were similar, as is clearly shown by the log $A$ versus $x$ plots of a granule cell (cell 964) and a CA1 pyramid cell (cell 503; Fig. 15). This raises the possibility of functional similarities between dendritic fields in these different cell classes.

Because of this possibility, Fig. 14 compares granule cell dendrites with the basilar dendrites of CA1 and CA3c pyramidal neurons. Granule cells were physically significantly longer than the basilar dendrites of CA1 neurons, but their $L_{out}^{max}$ was not significantly different at DC, and it was only slightly larger at 40 Hz. Even though granule cells and the basilar dendrites of CA3c neurons had similar anatomic lengths, $L_{out}^{max}$ was significantly and substantially larger in

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**Figure 9.** Left: two-dimensional anatomic projection of a granule cell (cell 964). Right: $V_{in}$ and $V_{out}$ neurotropic figures at DC and 40 Hz. Granule cells are electrotonically more compact than either CA1 or CA3 neurons. The dendritic branches have nearly identical electrotonic lengths.

**Figure 8.** Log $A$ versus $x$ plots at DC for the $V_{out}$ and $V_{in}$ transforms of the CA3c neuron in Fig. 7. The four steep diagonals correspond to the cluster of proximal apical branches in Fig. 7. The slopes of the terminal branches and basilar dendrites depend on the direction of signal propagation, as in Figs. 4 and 6.
granule cells. This suggests a greater isolation of distal dendritic regions from potential changes at the soma in granule cells than in the basilar dendrites of CA3c neurons. This difference in electrotonic architecture may affect the spatial profile of voltage-dependent synaptic interactions in ways that are important for synaptic integration and plasticity.

Granule cells had significantly shorter $L_{\text{max}}$ than did the basilar dendrites of both CA1 and CA3c pyramidal neurons at both DC and 40 Hz (Fig. 14, D and E). This may be attributable to the loading effect of the apical dendrites in the two classes of pyramidal cells.

Electrotonic location of synaptic inputs

Functional consequences of the anatomic distribution of synaptic inputs can be inferred from the log $A$ versus $x$ plots. One hippocampal synaptic pathway of particular experimental and theoretical interest is the mossy fiber projection from granule cells to CA3 pyramidal neurons. These axons terminate on large, proximal spines that have been called thorns or excrescences (Blackstad and Kjaerheim 1961; Ramon y Cajal 1911). The location of these synapses indicates that they may be especially suitable for biophysical studies of vertebrate central excitatory synaptic transmission using voltage clamp (Xiang et al. 1994). Their size and location have led to suggestions that they may produce an exceptionally powerful excitation, so that activity in a single mossy fiber could drive a postsynaptic CA3 cell to fire a spike (Marr 1971; McNaughton and Morris 1987). This implies a possible role for mossy input as a “teacher” in a Hebbian-style mechanism for associative learning. Questions about the functional significance of thorns also have been raised because of their unusual morphology (Blackstad and Kjaerheim 1961; Chicurel and Harris 1992).

Gonzales et al. (1993) recently have examined the distribution of thorns on CA3 pyramidal cells. In the basilar dendrites they found thorns within 2–95 $\mu$m of the soma; in the apical dendrites, the range of distances was 3.9–161 $\mu$m. Our ongoing work suggests that the most proximal dendritic locations might be well space clamped by an electrode in the soma (Carnevale et al. 1994). Although the most remote thorns seem anatomically close to the recording electrode, how close are they electrotonically? To answer this question, we referred to the log $A$ versus $x$ plots to determine the greatest electrotonic distances at which thorns might occur in the basilar and apical dendrites. These distances provide worst-case estimates of the experimenter’s ability to measure synaptically generated signals and influence membrane potential in the dendritic shaft at the base of the activated thorns.

The largest DC $L_{\text{thorn}}$ was 0.033 in the basilar and 0.091 in the apical dendritic field. Therefore voltage transfer from the soma to the dendritic regions populated by thorns would be very efficient at low frequencies ($f < 5$ Hz). Each millivolt imposed at the soma would produce at least $e^{-0.033} \approx 0.97$ mV at the site of the most distant basilar thorn, and $e^{-0.091} \approx 0.91$ mV at the base of the most distant apical thorn. This means that it could be relatively easy to reach the reversal potential for even the most distal mossy fiber input by sustained depolarization of the soma. Furthermore, because of the directional reciprocity between voltage and current/charge transfer, a somatic voltage clamp will capture $\approx 91\%$ of the total synaptic charge generated by a mossy fiber input.

However, attenuation increased progressively with frequency, and at 40 Hz, the largest $L_{\text{thorn}}$ was 0.25 in the basilar and 0.61 in the apical dendrites. Now each millivolt at the soma produced as little as 0.78 mV in the basilar region and 0.54 mV in the apical region populated by thorns. Thus rapid fluctuations of membrane potential at the soma would be attenuated substantially by the time they reach the most distal thorns. Furthermore, synaptic current transients produced by the most distant thorns would be delayed.
broadened, and attenuated by comparison with more proximal inputs. This implies that biophysical studies of synaptic function must use strict selection criteria for acceptability of data, even when the synapses seem to have favorable anatomic locations.

What about voltage transfer from the thorns to the soma? For the moment, let us ignore the effects of axial resistance within the thorns themselves, which may produce significant potential gradients between active synaptic zones and the nearby dendritic shaft (Brown et al. 1988). Instead we consider just the attenuation of voltage from a dendritic site to the soma. At DC the greatest $L_{\text{thorn}}$ was 0.57 in the basilar and 0.54 in the apical dendrites, so a 1-mV dendritic signal would produce only 0.56–0.58 mV at the soma. The situation was far worse at 40 Hz, where the electrotonic distances were 2.33 and 2.27, respectively, corresponding to $<$0.1 mV of somatic depolarization per millivolt in the dendrite. Therefore excitatory postsynaptic potentials generated by synapses on the most distal thorns could be attenuated grossly by the time they reach the soma. That is, some synapses that are anatomically close to the soma may be electrotonically too remote for accurate measurements of synaptic potentials.

Inputs from the Schaffer collaterals to CA1 cells are far less amenable to accurate investigation of synaptic mechanisms with a somatic electrode. These synapses are distributed widely over the length of the apical dendrites, rather than being restricted to a narrow lamina adjacent to the soma (Ramon y Cajal 1911; Schaffer 1892). Even if care is taken to activate synapses quite close to the cell body, the situation is unfavorable because the biophysical and anatomic properties of CA1 neurons produce steeper spatial gradients than would occur in CA3 cells. We evaluated the electrotonic location of all CA1 apical synaptic sites in the same range of physical distances from the cell body as thorns are found in CA3 neurons. We found that the worst case DC $L_{\text{out}}$ and $L_{\text{in}}$ for a hypothetical synapse onto a CA1 neuron in this range of distances would be 0.39 and 1.27. These electrotonic distances correspond to attenuations of 0.67 and 0.28, which are noticeably worse than for the most distal mossy fiber synapse in a CA3 cell.

**DISCUSSION**

A new conceptual approach to linear electrotonus

Whether at the level of brain circuits or individual cells, the functional significance of anatomy and biophysics cannot be fully appreciated by considering either separately. Each body of information must be examined in the context of the other so that a combined understanding of both emerges. This is particularly true in the case of electrical signaling in neurons. Historically, experimental investigations of neuronal anatomy and biophysics have proceeded along separate lines with relatively few intersections. This was due partly to the difficulty of obtaining complete and accurate morphometric data and partly to the lack of computational horsepower to handle anatomically and biophysically accurate models. Theoretical analyses of electrotonus accordingly tended to be framed in terms that required unrealistic assumptions about anatomy (Jack et al. 1983; Rall 1977) or were altogether independent of it (Carnevale and Johnston 1982). Technological advances largely have eliminated these problems, and it is now possible to address the relationship between neuronal form and function in ways that require a new analytic approach capable of integrating realistic anatomic and biophysical data.

Such an approach is the transformation from anatomic to electrotonic space (Brown et al. 1992; Carnevale et al. 1999a; Tsai et al. 1993, 1994b), the primary tool that we used in this comparative analysis of electrotonus in hippocampal principal neurons. The conceptual basis of this mapping is drawn from the work of Carnevale and Johnston (1982), who introduced the use of two-port network theory to the study of electrical signaling in neurons. At the core of prior approaches to linear electrotonus was the definition of electrotonic length as the ratio of physical distance to the length constant of an infinitely long cylindrical cable. Although this definition is convenient and appropriate for infinite cylindrical cables, it is cumbersome and confusing when applied to real neurons with their finite, irregularly branched dendritic trees. Two-port theory focuses instead on the fundamental problem of electrotonus: how efficiently do electrical signals spread within a cell?

The electrotonic transform builds on this basic idea, providing a conceptual framework for organizing a large body of anatomic and biophysical data and presenting it in forms that make functional implications readily apparent. The transform introduces two new analytic strategies. The first is to translate attenuation into a metric for signal loss by taking its logarithm. The second is to present this metric in two complementary graphic renderings: neuromorphic figures that preserve the branched architecture of the cell, and $\log A$ versus $x$ plots that emphasize the dependence of attenuation on physical location within the dendritic tree. The neuromorphic figures are particu-
larly useful for conveying an overall impression of the qualitative aspects of signal propagation throughout the dendritic tree. The log A versus x plots are especially helpful in the quantitative analysis of synaptic efficacy and amenability to study under voltage clamp. In the present investigation, both of these graphic presentations have been of value.

*An efficient algorithm for computing attenuations*

To make practical use of this new analytic approach, we had to develop an efficient program to calculate the attenuations. Existing simulation programs that compute time-domain solutions were unsuitable because of excessive run time, which was aggravated by the need for a separate run to calculate the $V_{in}$ attenuations from each of the terminal dendritic branches. Therefore we created a special program that uses an efficient algorithm to achieve $O(N)$ run times, computing attenuations with speed and accuracy that are independent of frequency.

This program operates in the frequency domain using a recursive algorithm. It generates the $V_{in}$ attenuations for all
terminal branches in a single pass instead of making a separate run for each branch. The tree structure of a neuron lends itself quite naturally to recursive algorithms of the kind we used. Other approaches are possible, such as sparse matrix methods, but we were disinclined to resort to them because they required forcing the dendritic tree into awkward data structures and needed greater effort to minimize storage and run time.

One special feature that further increases computational speed and accuracy is the representation of each segment of the cell by an equivalent T circuit whose elements are complex impedance functions. By doing this, we avoid drawbacks inherent in the conventional method of lumping the electrical properties of axoplasm and membrane into discrete resistive and capacitive elements. The conventional “lumping” approach requires progressively smaller compartment size to maintain accuracy at frequencies above \( f_m = 1/2\pi\tau_m \) (2.3–5.3 Hz in principal neurons of the hippocampus). This increases the number of compartments and the run time needed to calculate the attenuations. We derived the impedance functions of the equivalent T circuit from the impulse responses of a finite cylindrical cable (Tsai et al. 1994b). At all frequencies these functions are as accurate as the computer’s floating point precision, so compartment size and number do not have to be changed and run times are independent of frequency.

**General characteristics of electrotonus in hippocampal principal neurons**

This study revealed electrotonic regularities that transcend neuronal classifications. In a previous study of CA1 pyramidal neurons (Mainen et al. 1996), we reported that attenuation was worse for voltage spreading toward the soma (\( V_{in} \)) than away from it (\( V_{out} \)). In the present work, we confirmed this observation and extended it to CA3c pyramidal neurons and granule cells of the dentate gyrus. Furthermore, the proximal dendritic branches were the main feature of the \( V_{out} \) neuromorphic figures, whereas the distal branches dominated the \( V_{in} \) figures (Figs. 3, 5, 7, 9, and 11). Likewise, corresponding parts of the dendritic tree (basilar, primary apical, and distal branches) were associated with strikingly different slopes in the log \( A \) versus \( x \) plots for \( V_{out} \) and \( V_{in} \) (Figs. 4, 6, 8, 10, and 12). In particular, distal or terminal dendritic branches had similar slopes in the log \( A \) versus \( x \) plots; nearly flat for \( V_{out} \) and steep for \( V_{in} \). On the other hand, the log \( A \) versus \( x \) plots of proximal branches were steeper for \( V_{out} \) and flatter for \( V_{in} \). This demonstrates a general feature of electrotonus: attenuation depends strongly on the direction of signal propagation (Carnevale and Johnston 1982).

It also illustrates an important aspect of the electrotonic structure of these three cell classes. At DC and low frequencies, the membrane impedance is high compared with the axial resistance. When a signal propagates from the cell body into the dendrites, the axial current in each of the terminal branches (basilar or distal apical branch) is small because of their high membrane impedance and closed-end terminations. Consequently there is little voltage drop along the lengths of terminal branches. However, all the current that reaches these branches must first pass through proximal branches. This is the reason for the large voltage drop along the primary apical dendrite of CA1 cells and in the initial apical branch of granule cells that lie deep in the dentate gyrus. As frequency increases, the membrane impedance falls progressively, so that axial resistivity plays an even greater role and voltage attenuation becomes noticeable even in distal branches.

Signals propagating from the periphery toward the soma encounter a much different situation. Current passing through the axial resistance of a distal branch must supply not only the small amount of membrane that belongs to the branch but also the much greater amount of membrane that belongs to the larger caliber proximal branches and the soma. The relatively large axial resistance of a distal branch in series with the much lower impedance of the proximal branches and soma is analogous to a voltage divider, and results in severe attenuation along terminal branches even at DC and low frequencies.

Recently Korogod et al. (1994) presented an evaluation of electrotonus in rat abducens motor neurons, describing a tendency of dendrites to fall into groups with similar somatofugal voltage attenuations and spatial potential gradients in the steady state. We have not observed clustering of attenuations in principal neurons of the hippocampus. Furthermore, the spatial gradient was very similar in all terminal branches of any given cell, whether for DC or 40 Hz, somatofugal or somatopetal.

**Electrotonic differences between classes of hippocampal principal neurons**

Although CA1 and CA3c pyramidal cells are anatomically similar to each other, their electrotonic structures differ con-
siderably. First, CA1 cells have a primary apical dendrite that is revealed clearly and unequivocally by the transform. This architectural and electrotonic feature is notably lacking from the transforms of CA3c neurons, whose multiple apical branches appear to have roughly comparable electrotonic extents.

Transforms of CA1 neurons are also considerably larger than those of CA3c, particularly for $V_{out}$. Only part of this difference can be attributed to the slightly greater anatomic length of the apical field of CA1 cells. Most of it stems from differences in the branching patterns and membrane properties of these cell classes. Voltage drop along the primary apical dendrite is reflected in the higher attenuation in the $V_{out}$ transform of CA1 cells. As noted above, all the current that flows into the distal branches of these cells must pass through the primary apical dendrite, causing a large voltage gradient along its axial resistance. This resistive bottleneck, combined with the lower $R_m$ of CA1 neurons, ensures a larger $V_{in}$ transform. Because CA3c cells have multiple apical branches instead of a single primary apical, longitudinal current is divided among them so there is no resistive bottleneck. The primary apical dendrite of the CA1 cells guarantees an essential dissimilarity between the electrotonic architectures of these two cell classes that cannot be eliminated by any perturbation of $R_m$.

Of the three cell classes, granule cells are physically and electrotonically most compact, having $V_{out}$ and $V_{in}$ transforms with the smallest extent. Because the dendritic branching pattern of granule cells is far simpler than that of pyramidal cell apical dendrites, “electrotonic extent” in the broadest sense of the term is different even in those cases where the $L_{out}^\text{max}$ or $L_{in}^\text{max}$ are statistically indistinguishable from those of pyramidal cells.

An unexpected result of this study was the finding that there may be subtle anatomic variations among cells of a given class that have striking electrotonic effects. This was illustrated by the two granule cells, whose chief anatomic difference was in the presence (Fig. 9) or absence (Fig. 11) of an initial unbranched apical segment that lay between the soma and the proximal ends of the branches that constituted the dendritic “fan.” This neurite, which was barely noticeable in the anatomic images, had little or no effect on voltage transfer from dendritic synapses to the soma. However, it attenuated voltage signals spreading from the soma out to the dendrites by virtue of the axial resistance that it interposed between the soma and the remainder of the dendritic tree. This would reduce the effect of somatic events, including spikes, on the membrane potential at synaptic locations in the dendritic tree. Because of the reciprocal relationship between voltage and current transfer (Carnevale and Johnston 1982), we predict that this segment also would decouple synaptic currents generated in the dendritic tree from a voltage clamp attached to the soma, decreasing rise time and peak amplitude of the currents recorded by the clamp. Because the existence and length of this proximal segment depend on the location of the soma in the cell body layer of the dentate gyrus, the electrotonic structures of granule cells are not uniform. Instead they are distributed along a continuum that ranges from Figs. 9 and 10 at one extreme to features at least as pronounced as those of Figs. 11 and 12 at the other, depending on how deeply the soma lies in the in the cell body layer of the dentate gyrus.

Robustness of these results

How vulnerable are our findings to errors in the anatomic measurements and biophysical parameters from which the electrotonic transforms were computed? In METHODS, we noted that the morphometric data were obtained with a standard light microscope outfitted with a video camera (Claiborne 1992; Rihn and Claiborne 1990). Current work in our laboratories has found that confocal scanning laser microscopy can be used to improve the accuracy of diameter measurements (O’Boyle et al. 1993, 1996). This tends to reduce both diameter and surface area and consequently increases the input resistance of computational models of neurons. Furthermore, one might expect the greatest relative improvement of accuracy to be in the diameters of fine processes, which typically are distal from the soma in the three cell classes we studied. Our preliminary analyses of granule cells in the dentate gyrus indicate that this would increase attenuation both in the somatofugal and somatopetal directions (O’Boyle et al. 1993; 1996). While this would alter our quantitative findings, it would not affect the qualitative outcome of the work we present here.

Systematic errors in any of the biophysical parameters that applied roughly equally to each of the three cell classes might change the exact numeric values we report but would not alter the qualitative similarities within, nor the differences between, these classes. What about class-specific parameter errors? Most of the differences that we observed are both very significant ($P < 0.001$), with strong within-class clustering and between-class segregation, and numerically quite large (e.g., the comparisons between the apical fields of CA1 and CA3c cells). In a recent publication (Mainen et al. 1996), we explored the effects of a wide range of $R_i$ and $R_m$ values on the electrotonic architecture of CA1 pyramidal neurons. Those observations indicate that it would be unlikely for class-specific parameter errors as large as 20–30% to nullify any of the large differences we report here. However, differences between the basilar fields of CA1 and CA3c pyramidal neurons are smaller, so they might be more susceptible to class-specific parameter errors. It should be noted, however, that instead of obliterating a difference, an error could just as easily enhance it or even possibly reveal a previously unrecognized significant difference between classes.

Finally we must point out that attenuation at frequencies $>5f_m$ is determined almost entirely by $R_i$ and $C_m$, so errors in $R_m$ are likely to affect electrotonic structure only at DC and low frequencies. Therefore in these hippocampal neurons, only the DC and low frequency results will be susceptible to errors in $R_m$, whereas the 40 Hz results would be changed only by unreasonably large reductions of $R_m$ (five-fold or greater).

Effects of frequency on electrotonic architecture

Attenuation worsens markedly as frequency increases beyond $f_m$. Because of recent interest in synchronized 40
Hz activity in cortical neurons (Ahissar and Vaadia 1990; Eckhorn et al. 1988; Gray and Singer 1989; Gray et al. 1989; Loewel and Singer 1992), we examined the effects of frequency on signal propagation. To do this, we estimated the overall extent of the $V_{in}$ and $V_{out}$ transforms in log units by calculating the “tip-to-tip” lengths of the transforms as the sum of basilar and apical $L_{max}$ or $L_{tip-to-tip}$ for pyramidal cells or just the apical $L_{max}$ or $L_{tip-to-tip}$ for granule cells. Although the neurons we studied differ from neocortical cells, comparing these tip-to-tip lengths at 40 Hz against their DC values suggests the possible range of effects that frequency may have on electrotonic architecture.

At both DC and 40 Hz, CA1 neurons had the largest $L_{tip-to-tip}$ (1.08 and 3.2 log units) or $L_{tip-to-tip}$ (5.0 and 9.5). At DC, the $L_{tip-to-tip}$ of granule cells (0.15) was comparable with CA3c $L_{tip-to-tip}$ (0.23), but granule cells were electrotonically smallest by both measures at 40 Hz ($L_{tip-to-tip}$ 0.65, $L_{tip-to-tip}$ 2.3). The largest absolute increase of electrotonic extent from DC to 40 Hz was in CA1 pyramidal cells for $L_{tip-to-tip}$ (2.2) and in CA3c cells for $L_{tip-to-tip}$ (5.1). The greatest relative increase for $L_{tip-to-tip}$ was in CA3c cells (6.4 times). Granule cells and CA3c cells tied for the largest relative increase for $L_{tip-to-tip}$ (3.1 and 2.8 times, respectively). These results suggest that integration of high-frequency synaptic inputs in cortical pyramidal neurons occurs only over a very limited spatial range. Mechanisms for synchronization of firing therefore have to rely on synaptic inputs temporally from the amplitude and time course in the dendritic somatic measurements of postsynaptic potentials generated at nearby dendritic locations may differ substantially from those inputs that are too remote. The low-pass filtering effects of electrotonus suggest that rise time may be a useful indicator of the quality of voltage-clamp recordings, and we are evaluating criteria based on this approach (Carnevale et al. 1994).

**Effects of active and synaptic conductances on electrotonus**

As noted in METHODS, our computations of attenuation used values for the parameters $R_i$, $C_m$, and $R_m$ that reflect neuronal “small signal” properties (Mainen et al. 1996). That is, they include the contributions of both passive and active currents for a range of membrane potentials within a few millivolts of rest. What happens if membrane potential strays out of this range or if membrane properties are perturbed by synaptic conductances or pharmacological manipulations? Elsewhere (Mainen et al. 1996) we have discussed how active currents that arise in the soma and axon might affect electrical signaling in neurons (e.g., Stuart and Sakmann 1995); here we consider this question from a more general perspective.

Conductance changes that are localized in space and/ or time may, if large enough, introduce focal and/ or transient distortions of electrotonic architecture through localized alteration of the transmembrane flow of signal currents. It is difficult to anticipate all effects of voltage- and time-dependent conductances, whose dynamic properties can result in either attenuation or amplification of membrane potential fluctuations. However, it can be predicted that slow, sustained active currents, or tonic alterations of membrane properties as might be caused by background synaptic activity or application of channel blockers such as cesium, will affect attenuation primarily at DC and low frequencies. More than half of the transmembrane current is capacitative at frequencies above $f_m$ (see METHODS). Manipulations that decrease membrane conductance will not appreciably reduce attenuation at frequencies $>5f_m$; in this frequency range, $<20\%$ of the transmembrane current is ionic, so even completely blocking all ionic channels would have little effect. Furthermore, only a conductance increase that is much larger than the resting membrane conductance will alter the electrotonic architecture significantly at such high frequencies. Because $5f_m$ is $\sim12–25$ Hz in the hippocampal neurons we studied, their electrotonic architectures at frequencies in the range of 40 Hz will be relatively resistant to all but the most extreme changes of membrane conductance.

**Experimental accessibility of synaptic inputs to biophysical investigations**

The log $A$ versus $x$ plots provide a convenient tool for judging the accessibility of synapses to biophysical study via intracellular recording. In the RESULTS, we noted that somatic measurements of postsynaptic potentials generated at nearby dendritic locations may differ substantially from the amplitude and time course in the dendritic tree. On the other hand, by using log $A$ versus $x$ plots to interpret the previously described distribution of thorns on CA3c pyramidal cells (Gonzales et al. 1993), we found that many of the mossy fiber inputs onto these neurons are indeed close enough to the cell body for high accuracy measurement of synaptic currents under somatic voltage clamp. Even so, it will be necessary to apply carefully designed selection criteria to eliminate those inputs that are too remote. The low-pass filtering effects of electrotonus suggest that rise time may be a useful indicator of the quality of voltage-clamp recordings, and we are evaluating criteria based on this approach (Carnevale et al. 1994).

**Functional implications of morphological and biophysical changes**

The electrotonic transform already has been used to examine how the anatomic changes that accompany development affect the electrotonic architecture of neurons in the crayfish (Edwards et al. 1994; Hill et al. 1994). It could be used for a similar purpose in other species or to investigate the functional consequences of the alterations of neuronal anatomy and membrane properties that occur in the course of aging, disease, injury, and evolution or in response to the actions of neurotransmitters, neuromodulators, and drugs.

**Electrotonic transform as a basis for neuronal taxonomy**

Traditional approaches to neuronal classification have relied primarily or entirely on anatomic criteria. However,
anatomy is not an altogether reliable guide to the flow of signals in a cell. Our experience indicates that small and easily overlooked anatomical features, such as the initial apical stalk that is present on some granule cells, may have substantial effects on electrotonic architecture. Classifications based solely on anatomical characteristics may ignore easily overlooked but functionally important features. The electrotonic transform, which integrates anatomical and biophysical properties, can be used as the foundation of a new classification scheme that interprets the consequences of cellular anatomy for neuronal signaling. Such a functional reinterpretation of cellular anatomy may lead to a better understanding of the circuitry of the brain.

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REFERENCES


