Mechanisms of Electrical Coupling Between Pyramidal Cells

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Vigmond, Edward J., Jose L. Perez Velazquez, Taufik A. Valiante, Berj L. Bardakjian, and Peter L. Carlen. Mechanisms of electrical coupling between pyramidal cells. J. Neurophysiol. 78: 3107–3116, 1997. Direct electrical coupling between neurons can be the result of both electrotonic current transfer through gap junctions and extracellular fields. In vitro. Gap junctional electrotonic connections produced waveforms resembling small-amplitude excitatory postsynaptic potentials. Intracellular recordings from CA1 pyramidal neurons of rat hippocampal slices showed two different types of small-amplitude coupling potentials: short-duration (5 ms) biphasic spikelets, which resembled differentiated action potentials and long-duration (>20 ms) monophasic potentials. A three-dimensional morphological model of a pyramidal cell was employed to determine the extracellular field produced by a neuron and its effect on a nearby neuron resulting from both gap junctional and electric field coupling. Computations were performed with a novel formulation of the boundary element method that employs triangular elements to discretize the soma and cylindrical elements to discretize the dendrites. An analytic formula was derived to aid in computations involving cylindrical elements. Simulation results were compared with biological recordings of intracellular potentials and spikelets. Field effects produced waveforms resembling spikelets although of smaller magnitude than those recorded in vitro. Gap junctional electrotonic connections produced waveforms resembling small-amplitude excitatory postsynaptic potentials. Intracellular electrode measurements were found inadequate for ascertaining membrane events because of externally applied electric fields. The transmembrane voltage induced by the electric field was highly spatially dependent in polarity and wave shape, as well as being an order of magnitude larger than activity measured at the electrode. Membrane voltages because of electrotonic current injection across gap junctions were essentially constant over the cell and were accurately depicted by the electrode. The effects of several parameters were investigated: 1) decreasing the ratio of intra to extracellular conductivity reduced the field effects; 2) the tree structure had a major impact on the intracellular potential; 3) placing the gap junction in the dendrites introduced a time delay in the gap junctional mediated electrotonic potential, as well as decreasing the potential recorded by the somatic electrode; and 4) field effects decayed to one-half of their maximum strength at a cell separation of ~20 μm. Results indicate that the in vitro measured spikelets are unlikely to be mediated by gap junctions and that a spikelet produced by the electric field of a single source cell has the same waveshape as the measured spikelet but with a much smaller amplitude. It is hypothesized that spikelets are a manifestation of the simultaneous electric field effects from several local cells whose action potential firing is synchronized.

INTRODUCTION

Electrical coupling of cells has been proposed as a factor in spike entrainment of epileptiform bursts (Carlen et al. 1996; Dudek et al. 1986). This type of coupling works through two distinct mechanisms, gap junctions and extracellular fields. Gap junctions are channels that directly link the cytoplasm of two cells, providing a low-resistance pathway, as well as allowing diffusion of small molecules (Dermietzel and Spray 1993). Although both types of coupling mechanisms are known to be present in the brain (Dudek et al. 1986), the relative contribution of each is not known. There have been observations of intracellularly recorded small-amplitude waveforms that seem to be the product of coupling (MacVicar and Dudek 1981; Taylor and Dudek 1982; Valiante et al. 1995). Waveforms that have amplitudes ~5 mV, are biphasic, and have a duration comparable with that of an action potential, are referred to as spikelets. They are particularly interesting because they appear to be first derivatives of action potentials (Valiante et al. 1995), which is indicative of field coupling (Bardakjian and Vigmond 1994). Furthermore, it was observed that measures that increased neural synchrony, presumably by enhancing gap junctional conductance, led to increased spikelet frequency but without a change in amplitude. Similarly, it was observed (Perez Velazquez et al. 1994) that the synchronization of neural activity in hippocampal slices in Ca2+-free perfusate as measured by field potentials was abolished by putative gap junctional blockers. Manipulations that caused intracellular alkalinization, which has been shown to increase dye coupling between neurons (Church and Baimbridge 1991), resulted in increased synchronization and, conversely, blocking gap junctional mechanisms abolished synchronized epileptiform potentials and decreased dye coupling. These observations suggest that gap junctions play a role in synchronizing action potential firing in the slice. The relative role of each type of coupling, direct electrotonic transfer of current across a gap junction or field effects between neurons, in producing spikelets is unknown at this point.

Computer simulations were performed to model electrical interaction between two neurons. The boundary element method (Vigmond and Bardakjian 1996) was used with a novel implementation, employing a mixture of triangular somatic elements and cylindrical dendritic elements to couple three-dimensional models of neurons. Previous studies have modeled extracellular dendritic fields (Klee and Rall 1977; Rall 1962, 1969; Zucker 1969) but have relied on simplified geometry. In addition, these studies have not computed the effects of these fields. In this study, assuming an active source cell, the effects of gap junctions and extracellular fields on transmembrane voltage and intracellular potential in a neighboring passive cell were computed. These
results were compared with biological data and a generation scheme for spikelets was hypothesized.

METHODS

Boundary element method

The boundary element method is a numerical technique that allows the solution of Laplace’s equation over a volume by solving for quantities on the surface that bounds the volume (Beer 1992). The surface is discretized into a contiguous set of elements to enable integration over the surface. In these simulations, Laplace’s equation is solved for the intra- and extracellular electric scalar potentials. This required the evaluation of the electric potential and its normal derivative on the cell membranes, as well as boundary conditions relating the intra- and extracellular domains. The normal derivative of the potential is a scaled version of the transmembrane current.

Two model neurons were placed next to each other in an infinite medium. One cell was considered active and called the source (s) cell and the other was passive and called the receiving (r) cell. The effect of the receiving cell on the source cell was ignored as it has been shown to be small (Vigmond and Bardakjian 1995). The transmembrane voltage of the source cell was assigned by using an intracellular recording from a CA1 neuron that was smoothed with a five-point moving average window. The recording described a surface depolarization wave that was assumed to start at a specific spot on the membrane and travel with a constant propagation velocity (0.1 m/s) over the surface of the soma, into the dendrites (Stuart and Sakmann 1994).

Pulse functions were used for each element meaning that any quantities associated with an element were constant over the element (i.e., quantities that occur intracellularly; e.g., those occurring extracellularly). The resulting matrix equations relating the scalar potential at the surface of a cell, $\phi$, to the transmembrane current density, $i_m$, can be expressed (Leon and Roberge 1990; Vigmond and Bardakjian 1996)

\[
\begin{align*}
\left( \frac{I}{2} - H \right) \phi_s &= \frac{1}{\sigma} G_{i_m} + \phi^e_s \\
\left( \frac{I}{2} + H \right) \phi_r &= -\frac{1}{\sigma} G_{i_m} + \phi^r_s \\
v_m &= \phi_r - \phi_s \\
i_m &= C_m \frac{\partial v_m}{\partial t} + g_m v_m
\end{align*}
\] (1-4)

where $v_m$ is the vector of transmembrane voltages, $G$ and $H$ are matrices describing the effect of single- and double-layer sources respectively, $\sigma$ is the conductivity, $C_m$ is the diagonal matrix of elemental membrane capacitances (F/m²), $g_m$ is the diagonal matrix of elemental membrane conductances (S/m²), $I$ is the identity matrix, and $\phi^e_r$ is the potential resulting from an applied current source that may be intracellular or extracellular in origin as the result of gap junction or fields, respectively. The conductivity of the intracellular and extracellular media were initially set to 133 S/m. Note that membrane properties need not be uniform.

The boundary element method (BEM) assumes that 1) the intra- and extracellular media are homogeneous and isotropic; 2) the system is quasistatic, i.e., the electric and magnetic fields can be decoupled; and 3) the initial conditions are known for all state variables. As long as these three conditions are met, any relationship relating transmembrane voltage to current density may be used (Leon and Roberge 1990), not necessarily a linear one. Because of the small voltage perturbations being studied and the increased computation time for more elaborate equations, a linear membrane model was used to determine the transmembrane currents from voltages in Eq. 4 by using constant membrane conductances.

A Galerkin formulation was used because solution at only one point of the cylindrical elements provided inaccurate results, especially for the diagonal terms involving branch elements. Analytic expressions for the computation of potential for triangular elements were derived (Vigmond and Bardakjian 1996) and the expressions for cylindrical elements are given in APPENDIX. To generate $G$ and $H$, these expressions were integrated over the field point element by Gaussian quadrature (Press et al. 1994).

By algebraic manipulation of Eqs. 1-4, it was possible to calculate $\phi^e_r$ and $i_m$ knowing $v_m$ because the effect of the receiving cell was ignored. The potential induced on the extracellular surface of the receiving cell by electric fields generated by the source cell is given by

\[
\phi^e_r = H^0 \phi^e_s + \frac{1}{\sigma} G^0 i_m
\] (5)

Here, $H^0$ and $G^0$ are the matrices representing the extracellular field coupling. They were computed in a manner similar to the single- and double-layer matrices.

Gap junctions were represented as intracellular current sources and were modelled as simple resistors linking the two cells. The current flow through the gap junction was the difference in intracellular potentials of the receiving and source elements linked by the gap junction, multiplied by the conductance of the junction, $g_j$. The influence of the gap junction was then seen as an applied intracellular current. Assuming element $m$ of the receiving cell was connected to element $n$ of the source cell through the gap junction, the effect at another element, $l$, was able to be computed from

\[
(\phi^l)_n = \frac{g_j}{A_m} [(\phi^r_i) - (\phi^s_i)] (G^l)_{i m}
\] (6)

where $A_m$ is the area of element $m$ of the receiving cell, $(\cdot)_{i}$, refers to the $i$th array entry and $(\cdot)_n$, refers to the matrix entry in the $n$th column of the $i$th row.

After some manipulation, the solution of the intracellular surface potential from the receiving transmembrane voltage and source potentials and currents is as follows

\[
\left[ 1 + \frac{\sigma}{\sigma_i} \right] \frac{I}{2} \left[ 1 - \frac{\sigma}{\sigma_i} H - \frac{g_j}{\sigma_i} G^i \right] \phi^i_l = \frac{\sigma}{\sigma_i} \left[ \frac{I}{2} - H \right] v_m + \phi^e_r - \frac{g_j}{\sigma_i} G^i \phi^e_l
\] (7)

where $G^i$ is $G^e$ with all entries except the $m$th column set to zero. Knowing $\phi^r_i$, Eq. 2 was used to determine $i_m$.

The receiving cell was assumed to be passive and its membrane was modeled as a resistive-capacitive (RC) network with the ionic current modeled as a simple conductance. Having knowledge of $i_m$, the rate of change of $v_m$ could be computed from Eq. 4. Thus a system of first order differential equations was constructed that solved for the receiving transmembrane voltage given the source transmembrane voltage. A variable step-size integrator employing Gear’s method (Radhakrishnan and Hindmarsh 1993) was used to solve the system.

The potential recorded from an intracellular electrode, $\phi^i_l$, can be computed once $\phi$ and $i_m$ are known by application of Green’s theorem (Bardakjian and Vigmond 1994). The field point is taken to be the center of the cell and it follows that

\[
\phi^i_l = -H^0 \phi^e_s - \frac{1}{\sigma} G^0 i_m
\] (8)

where $G^e$ and $H^e$ are the contributions of the monopoles and dipoles to the electrode potential, respectively.
Model neuron

To model a neuron by the BEM, an accurate three-dimensional representation of the cell including its dendritic tree is needed. By using data from CA3 neurons (Major et al. 1994), such a model was constructed (Fig. 1). The soma was represented as a cylinder that slightly tapered near the ends, with a central radius 6 µm and a length of 79 µm. A total cell length of 400 µm was used. Membrane capacitance was set to 1 µF/cm². The somatic conductance was 0.4 S/m² and the dendrites were assumed to have a 0.17 S/m² conductance (Wright et al. 1996). These conductances gave a time constant of 61 ms for the entire system and a whole cell resistance of 100 MΩ, values that are close to those measured (Major et al. 1994). Parameter values are summarized in Table 1.

The entire dendritic tree was not replicated but the physical length and diameter of several branches were consistent with the biological data. Both basal and apical branches were considered. To imitate the effect of an entire tree, the effective dendritic capacitance and conductance were appropriately scaled, as is done to incorporate the increased surface area resulting from dendritic spines (Rapp et al. 1993). If F is the ratio of the spined branch area to the unspined branch area and BN is the density adjustment, the ratio of actual branches to model branches, both the conductance and capacitance are multiplied by F × BN. A value of two was used for F and N B was set to three. The axon was ignored because it is very small compared with the dendrites, having a diameter one-third that of a terminal dendritic branch (Stuart and Sakmann 1994). It is lost within the large tree of the dendrite, adding very little to the extracellular field in that region. After adjustment, the cell surface area was 74,900 cm², with 69 dendritic tips.

The surface of the cell was discretized into a set of contiguous elements. The somatic surface was discretized into a set of 96 triangles. Such a strategy applied to the dendrites would have lead to an unwieldy number of elements. Thus to reduce the number of elements in the trees, cylindrical elements were employed. Cylindrical elements have been previously employed to model cardiac cells (Hogues et al. 1992; Leon and Roberge 1994) but a radially symmetric field had been assumed. Here, it is assumed that the diameters of the dendrites are small enough that potential variation from one side to the other may be neglected. Because the soma had a much larger diameter, substantial gradients in potential may exist circumferentially precluding the use of cylindrical elements. Three types of cylindrical elements were used to build the dendritic trees: open ended cylinders, cylinders with one closed end for terminal segments, and branch points comprised of three open ended cylinders in a y-configuration (Fig. 1). A total of 454 elements were used to model the dendrites. The number of elements was chosen such that convergence in the solution was reached, i.e., more elements did not lead to an appreciable change in solution, while at the same time the number of elements was minimized.

When triangles and cylinders are used in the same boundary element model, there will be an interfacial cylinder that will join the two element types (Fig. 1A). Thus there must be an opening in the triangularly tessellated section that approximates a circle. Regardless of how many triangles are used, the opening will never be truly round and flux will escape through the gap between the cylinder and straight line segments defining the opening. This effect is insignificant for monopolar calculations but not for the dipolar calculations. In the latter case, the interfacial cylindrical element must be treated specially. The end away from the triangular elements was treated normally but the cylinder end at the junction of

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**TABLE 1.** Cell parameters for model neuron

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic conductance ( g_s )</td>
<td>25 ( \text{kS/cm}^2 )</td>
</tr>
<tr>
<td>Dendritic conductance ( g_d )</td>
<td>58.8 ( \text{kS/cm}^2 )</td>
</tr>
<tr>
<td>Membrane capacitance ( c_m )</td>
<td>1 ( \mu \text{F/cm}^2 )</td>
</tr>
<tr>
<td>Cell length</td>
<td>400 ( \mu \text{m} )</td>
</tr>
<tr>
<td>Cell area*</td>
<td>74,900 ( \mu \text{m}^2 )</td>
</tr>
<tr>
<td>Number dendritic tips*</td>
<td>69</td>
</tr>
<tr>
<td>Intracellular conductance ( \sigma_i )</td>
<td>133 ( \text{S/cm} )</td>
</tr>
<tr>
<td>Extracellular conductance ( \sigma_o )</td>
<td>133 ( \text{S/cm} )</td>
</tr>
<tr>
<td>Density adjustment ( N_B )</td>
<td>3</td>
</tr>
<tr>
<td>Spined/unspined area ( F )</td>
<td>2</td>
</tr>
<tr>
<td>Propagation velocity</td>
<td>0.1 ( \text{m/s} )</td>
</tr>
<tr>
<td>Time constant</td>
<td>61 ms</td>
</tr>
<tr>
<td>( R_s )</td>
<td>108 ( \text{MΩ} )</td>
</tr>
</tbody>
</table>

* Denotes values incorporating spine and density adjustment as defined in METHODS.
the two types was treated as a set of triangular elements, not a disc. This remedied the geometrical mismatch between the triangles and cylinder by closing any gaps at the interface.

To determine the dendritic segmental diameters, the terminal trode is given in Fig. 3. In the absence of gap junctions, the recorded potential was a biphasic waveform, lasting as long as the action potential with a peak amplitude of 80 µV. As the gap junction conductance was increased, the recorded potential increased in amplitude and seemed to be composed of two components: a monophasic potential that rose for the duration of the source action potential and then decreased and the biphasic waveform, which did not change with conductance. As the gap junction conductance was increased, the monophasic potential reached a higher value but also decayed slightly more rapidly. This was the result of increased shunting of current through the gap junction in addition to the transmembrane flow. The field effects were always present, superimposed on the gap junction effects. As the gap junction conductance increased, the field effects became insignificant. Integrating the field-induced potential with respect to time did indeed produce a waveform that was very similar to the source action potential (Fig. 4). By contrast, integrating the gap junction electrotonic potentials did not produce a waveform that resembled the source action potential.

The transmembrane voltage is the quantity of interest that will affect voltage-dependent membrane processes such as ion channel activity. The transmembrane voltage may be relatively uniform or differ drastically over the surface of the cell depending on whether or not the coupling is primary.

### Brain slices and solutions

Wistar rats (20–30 days old) were anesthetized with halothane (Fluothane, Ayerst Laboratories, Montreal, Canada) and decapitated. Transverse brain slices (400 µM) were obtained with a Vibratome (Series 1000, Technical Products International) and maintained in artificial cerebrospinal fluid (ACSF) that contained (in mM) 125 NaCl, 5 KCl, 1.25 NaH_{2}PO_{4}, 2 MgSO_{4}, 2 CaCl_{2}, 25 NaHCO_{3}, and 10 glucose, pH 7.4 when aerated with 95% O_{2}-5% CO_{2}. Osmolarity was 300 ± 5 (SE) mOsm. Calcium free ACSF was the same but without added CaCl_{2} and with 1 mM EGTA to reduce contaminating calcium. The internal solution in the recording electrode contained (in mM) 150 potassium gluconate, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 2 Mg-ATP, and 5 KCl, pH 7.2, adjusted with KOH, osmolarity 265 ± 5 mOsm.

### Electrophysiological recordings

For recordings, slices were transferred to a superfusion chamber maintained at 35°C (Medical Systems, Model PDMI-2). Neuronal recordings were performed with the whole cell configuration of the patch-clamp technique (Hamill et al. 1981). Patch pipettes were pulled from borosilicate capillary tubing (World precision Instruments, New Haven, CT). Electrodes had tip resistances ranging from 4 to 6 MΩ when filled with internal solution. Signals were filtered at 1 kHz, digitized at 88 kHz, and stored on video tape with a digital data recorder VR-10 (Instrutech, NY) for later playback and analysis.

For spikelet analysis, 60–120 s of neuronal activity were digitized at 10 kHz by using Fetchex (Axon Instruments). Spikelets were detected and analyzed with PCLAMP6 software (Axon Instruments).

### Results

#### Biological recordings

Perfusion of hippocampal pyramidal neurons in calcium-free ACSF causes cells to spontaneously fire and extracellular epileptiform field potentials are apparent (Perez Velazquez et al. 1994). Waveforms recorded by patch electrodes (Fig. 2) can be placed into three categories: 1) action potentials that may either appear singly or in bursts (Fig. 2A); 2) small-amplitude short duration potentials (spikelets) that are biphasic and last as long as an action potential, which also appear in bursts and singly (Fig. 2B); or 3) small-amplitude long-duration potentials that are monophasic and have an initial peak and a decay much longer than an action-potential duration (Fig. 2C).

### Gap junctional conductance

The simulated potential measured by an intracellular electrode is given in Fig. 3. In the absence of gap junctions, the recorded potential was a biphasic waveform, lasting as long as the action potential with a peak amplitude of 80 µV. As the gap junction conductance was increased, the recorded potential increased in amplitude and seemed to be composed of two components: a monophasic potential that rose for the duration of the source action potential and then decreased and the biphasic waveform, which did not change with conductance. As the gap junction conductance was increased, the monophasic potential reached a higher value but also decayed slightly more rapidly. This was the result of increased shunting of current through the gap junction in addition to the transmembrane flow. The field effects were always present, superimposed on the gap junction effects. As the gap junction conductance increased, the field effects became insignificant. Integrating the field-induced potential with respect to time did indeed produce a waveform that was very similar to the source action potential (Fig. 4). By contrast, integrating the gap junction electrotonic potentials did not produce a waveform that resembled the source action potential.

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ELECTRICAL COUPLING BETWEEN PYRAMIDAL CELLS

Fig. 3. Simulated effect of gap junction conductance on intracellularly recorded potential in receiving cell. Potential at center of cell is computed as a function of gap junction conductance for cells separated by 3 nm. Gap junction connects central regions of adjacent somata. Inset: an expanded view of initial depolarization. Waveforms in inset are similar to biologically recorded waveforms in insets of Fig. 2, B and C, except that amplitude of waveforms associated with 0 or low gap junction conductances are much lower than those of Fig. 2B.

Fig. 4. Relationship of received potentials to source action potential. A: source action potential is differentiated and scaled for comparison, with received potentials produced by gap junctions and field effects only. B: received potentials are integrated and scaled so comparison can be made with source action potential.

ibly gap junction or field-induced. The effects of field coupling on transmembrane voltage are shown in Fig. 5. The transmembrane voltage was a highly spatially dependent quantity. Depending on which point of the membrane was observed, the voltage was either depolarizing or hyperpolarizing and resembled either first or second derivatives. Also, the simulated transmembrane voltage was an order of magnitude higher than potentials measured at the somatic recording electrode. Hence, the intracellular or whole cell patch recording electrode could not accurately measure field coupling, nor did it represent what was happening across the neuronal membrane at different locations on the neuronal surface.

With gap junctional electrotonic coupling, the junctional current distributed itself evenly over the receiving cell and the transmembrane voltage was uniform across the cell (Fig. 6B). Furthermore, the transmembrane voltage and the intracellular potential were identical and the recording electrode accurately depicted the situation across the entire membrane.

Location of gap junction

Dendro-dendritic gap junctions have been found and may be more numerous than soma-somatic gap junctions (Dudek et al. 1986). Apical and basal dendro-dendritic connections were both investigated. Apical junctions at two electrotonic lengths (λ) from the soma, 0.25 and 0.43 λ, were simulated, as well as a basal junction located 0.13 λ from the soma. A gap junction with a conductance of 1 nS was used in each case. The results are plotted in Fig. 6. Moving the gap junction farther away from the soma reduced the electrode potential, smoothed out the waveform, and introduced a time delay as noted by Rall’s modeling of synaptic location on dendrite (Rall 1967).
FIG. 5. Transmembrane voltage as a function of position. With no gap junction, transmembrane voltage is computed at different points of soma: apical, at base of apical dendritic tree; central, at midpoint of soma; and basal, at base of basal dendritic tree. Note higher amplitude of these potentials compared with intracellularly measured potentials of an adjacent neuronal spike (e.g., Fig. 4A).

The effects on \( V_m \) were the same as those on \( \phi^E \). In Fig. 6B, a major difference between gap junctional and field coupling can be seen. Initially, field effects dominated and different points on the surface of the cell experienced different transmembrane voltages. As the field effect died off and the gap junction effect grew, the cell became more isopotential as the gap junction current distributed itself rather uniformly along the cell membrane. Initially, the potentials from each site depended on position but merged into one curve as time progressed.

Separation

The effect of distance on the received potential is seen in Fig. 7. The transmembrane voltage induced in a cell decreased as the cells were separated, with the voltage decreasing to one-half maximum at 27 \( \mu \text{m} \). Regression fitting to an exponential function yielded a decay rate for the intracellular potential that was approximately the inverse of the distance, \( r \) (actually \( r^{-1.03} \)). The transmembrane voltage decayed at a similar rate because it was linearly related to the electrode potential. It should also be noted that the peaks of the received potentials all occurred at the same time irrespective of distance.

Electrode placement

The recording electrode has been assumed to be at the center of the cell. Generally this will not be the case when making biological recordings. For patch electrodes, the electrode is usually somewhere on the soma or large proximal dendrite. The effect of different receiving measuring sites within the soma was considered. The intracellular potential was sampled at various points along the axis of the soma. Waveforms from the various positions were indistinguishable (data not shown). Because the intracellular conductivity was large, the potential did not vary greatly within the cell and exact electrode placement seemed to be irrelevant.

Trees

The effect of the dendritic trees on the receiving potential was determined in the absence of gap junctions (Fig. 8). The source cell remained unchanged with both apical and basal trees and the receiving cell had either no trees, only one tree, or two trees. Depending on the tree structure, a very different potential was observed. With the apical tree
only, the receiving potential was reversed in polarity from the control case of two trees. The potential occurred slightly sooner and was larger in magnitude as well. With only the basal tree, the received potential was triphasic. The initial portion was hyperpolarizing, followed by a larger depolarizing portion and finally a smaller and longer lasting hyperpolarization. It resembled the negative derivative of the two tree case, making it the negative second derivative of the source action potential. Finally, with no trees, i.e., a slightly tapered cylinder, the electrode potential again resembled a second derivative although reversed in polarity from the basal tree case and slightly increased in amplitude. Thus depending on tree structure, the electrode measured positive or negative first or second derivatives of the source action potential.

**Extracellular conductivity**

Decreasing the conductivity ratio \( (\sigma_i/\sigma_e) \) decreased the electrode potential and transmembrane voltage (Fig. 9). The effect was to simply scale the waveforms. Assessing the effective conductivity of the extracellular medium is difficult because even though the specific conductivity of the extracellular medium is known, the effect of the restricted extracellular space must be considered. Dense packing of neurons and glia such as occurs in dentate gyrus (DG) and pyramidal layers of the hippocampus lead to a reduction of the effective conductivity as the tortuosity is increased.

**Discussion**

Electrotonic gap junctional coupling is very different from field coupling. Field coupling is briefer in duration, depolarizing some regions of the membrane while simultaneously hyperpolarizing other regions and producing waveforms resembling first and second derivatives of the source action potential. Furthermore, intracellular recordings represent a bulk recording that is very different from the membrane electrical events. Transmembrane events, resulting from field potentials, are an order of magnitude larger than what are measured by the intracellular recording electrode. For events mediated through the gap junction conductance, the intracellular recording electrode conveys an accurate representation of transmembrane events because the current injected through the gap spreads itself out rather uniformly along the membrane. Field effects do not inject current into the receiving cell, but rather cause a redistribution of charge along the intracellular and extracellular surfaces of the membrane. This accounts for the very quick time constant of the electrical activity caused by this type of coupling as charge does not have to flow through the membrane, which has a time constant on the order of tens of milliseconds. An additional consequence is that field effects are independent of the receiving cell voltage and appear superimposed on top of any voltage present.

Gap junctional coupling induces potentials in the receiving cell that last longer than the duration of the action potential in the source cell for several reasons. Current will continue to flow through the gap junction as long as the intracellular potential of the source cell is higher than in the receiving cell. This condition is met during almost all of the action potential because the depolarization in the receiving cell is only a couple of millivolts. Although gap junctions are modulated by voltage, the opening and closing time constants are on the order of seconds (Moreno et al. 1994), a time scale two orders of magnitude larger than the one with which we are dealing. Hence, the static resistor is an acceptable model when only one action potential is being considered. The membrane voltage of receiving cell will decay passively with a time constant on the order of 15 ms, again longer than an action potential, with the gap junctional potential appearing like an excitatory postsynaptic potential (EPSP). Hence, as spikelets are observed to only last as long as the action potential, it seems unlikely they are mediated electrotonically through gap junctions whose effects last longer than the duration of an action potential.

Another possible source of differentiation of transmembrane voltage is the capacitance of the gap junctional aggregate. Assuming there is no conductance between membranes in the region of the aggregate, a capacitor is formed with a unit area capacitance one-half that of only one membrane. By using the recorded action potential to determine the derivative and taking an area of 0.28 \( \mu \text{m}^2 \) for the aggregate (Dudek et al. 1983), the current would be on the order of 0.2 pA (Fig. 10). This is 1/500 of the current flowing through a 1-nS conductance. Note that this is an overestimation because the capacitor is leaky and current will be shunted into the extracellular medium. Hence, this current is too small to cause spikelets.

The intracellular potential calculated in the receiving cell is consistent with spikelets in both shape and duration when
the gap junction is ignored. However, the simulated amplitude is much smaller than that measured in vitro. This suggests spikelets are the product of several cells firing synchronously if electric field effects are to be responsible for spikelet generation. This is in agreement with the conclusions of previous studies of hippocampal CA1 neuronal firings (Anderson 1971; Herreras et al. 1987), which suggested functional aggregates of three to five neurons during measured population spikes in vivo.

The potential produced by a single cell is small, below the noise level of the recording equipment. Individual events will go unnoticed. To reach a measurable level there must be a synchrony of several cells. On the basis of decay with distance, synchronously firing neurons three soma widths away would still have a significant effect compared with adjacent neurons. Gap junctions could mediate this synchrony as they provide a pathway for almost instantaneous coupling. This is consistent with the observations of pharmacological manipulations, which altered gap junction conductance (Perez Velazquez et al. 1994). Increasing the conductance through intracellular alkalinization would lead to a greater probability of action-potential propagation across the gap and hence, greater synchrony. Conversely, intracellular acidification or application of octanol will lead to a loss of synchrony through a decrease in gap junction conductance (Perez Velazquez et al. 1994; Valiante et al. 1995). It is to be stressed that the cell in which the spikelet recording is being made does not have to be electrotonically coupled to any other cell and is not coupled electrotonically to the synchronously firing adjacent neuronal aggregate that produces the field causing the spikelet in the recorded neuron. Furthermore, depolarizing spikelets might, by themselves, be a significant synchronizing influence between adjacent neurons or neuronal aggregates. Note that because spikelet potentials are biphasic, a small time delay between neighbouring firing neurons can lead to one cell producing a depolarizing pulse while the other produces a hyperpolarizing pulse, diminishing the combined result.

The pyramidal cell layers display a highly laminar structure. The neurons are organized in a strip with the somata in a thin layer and all trees oriented in the same direction. This is ideal for field coupling as electric fields produced by the neurons will be oriented in the same direction and the resultant field a simple superposition. A firing cell may be considered a dipole source oriented in the direction of action-potential propagation over the cell surface. If the cells were randomly placed, the spatial dependence of the dipole fields would lead to cancellation and synchrony would be meaningless. The laminar structure of the CA1 or CA3 regions leads to greater field effects.

Results of this study suggest a role for both types of electrical coupling in the hippocampus. Individual neurons induce intracellular potentials in neighbouring cells through extracellular fields that are too small to measure. There exist aggregates of neurons whose electrical activity is possibly synchronized in part by gap junctions. Such synchronized behavior produces fields that are the superposition of the fields produced by the individual neurons and result in measurable intracellular potentials in nearby cells. These field-induced potentials may cause cells near threshold to fire and bring about entrainment, exacerbating or inducing epileptiform activity in the slice.

APPENDIX: A DERIVATION OF FORMULAS FOR MATRIX ENTRIES

The formulas developed are applicable to zeroth order boundary elements, meaning that any quantities associated with an element are constant over that element. A matrix entry in column l of row k describes the potential produced by the lth element on the kth element. There are two types of sources to consider: monopoles and dipoles. The double layer potential calculation for zeroth order elements is equivalent to the calculation of the solid angle divided by 4π. For a closed surface, the solid angle is zero for points outside the surface, 4π for points inside, and 2π for points on continuous portions of the surface (Fig. A1). A nonclosed surface may be considered as a closed surface with a negative surface defining the opening. Therefore the solid angle for an open surface can be evaluated directly from the original open surface or by taking the negative of the result for the negative surface. The potential produced by a uniform distribution of dipoles on element l with its area defined by Πl at a point on element k is given by
(H)_{ij} = \int \int \int_{V_{ij}} \frac{r \cdot \overrightarrow{nd} \overrightarrow{r}^t}{4\pi |r|^3} \quad (A1)

where \( n \) is the outwardly directed normal of the source element and \( r \) is the distance vector from the point on the source element to the field point.

The potential produced by a uniform distribution of monopole sources is

\[(G)_{ij} = \int \int_{\epsilon_i} \frac{\overrightarrow{d}^t \overrightarrow{r}^t}{4\pi |r|^2} \quad (A2)\]

**Disc elements**

Assume the field point is at the origin and a disc of radius \( r \) is perpendicular to the \( z \) direction with its center at \((\rho_0, 0, z)\). In cylindrical coordinates, the integral can be expressed

\[(G)_{ij} = \int_{\rho_0}^{\rho} \int_{0}^{\pi} \frac{\rho \cdot \overrightarrow{d} \overrightarrow{p} \overrightarrow{\phi}}{4\pi \sqrt{\rho^2 + z^2}} \quad (A3)\]

Referring to Fig. A2, we see that the limits on \( \rho \) and \( \phi \) depend on whether or not the \( z \) axis intersects the disc. Unfortunately, because of the complicated nature of the limits expressed, further analytic integration is not possible and Gaussian quadrature is required.

**Cylinder with one closed end**

For monopole calculations, the element may be treated as a disc and an open cylinder. For dipole calculations, it is the negative of the result for the disc defining the open end.

**Branches**

Branches are simply treated as three separate cylinders that share branches as follows is used on the opposite end (Fig. 2). For monopolar calculations, the element may be treated as a disc and an open cylinder. For dipole calculations, it is the negative of the result for the disc defining the open end.

\[
\alpha = \frac{1}{2} \tan \frac{\alpha}{2} \sin \beta \quad (A7)
\]

\(\alpha \) and \( \beta \) will represent the direction of largest and smallest angular spread, respectively. Again, any cross section of this scaled cone will have the same solid angle. Thus the problem of finding the solid angle of a disk can be reduced to one of finding the cone whose cross section when cut by a \( z \) plane is circular (see Fig. A2).

Without loss of generality, the center of the disk may be considered to lie on the \( y = 0 \) plane at coordinates \((x, 0, z)\). We must now construct a right elliptical cone around the disk. Points on the disk corresponding to the direction of greatest spread of the cone are

\[r_{AS} = (x + \rho, 0, z)\]  and  \[r_{AE} = (x - \rho, 0, z)\]  \( (A8) \)

and points corresponding to the direction of smallest spread are

\[r_{AS} = (x, \rho, z)\]  and  \[r_{AE} = (x, -\rho, z)\]  \( (A9) \)

Cylindrical elements

For a cylindrical element with two open ends, the monopole entry is done in a straightforward manner. The openings are located at \( z_1 \) and \( z_2 \) with equal radii of \( r \) and the axis of the cylinder is located \( r_0 \) away from the \( z \)-axis. For a point at the origin, the distance vector to a point on the cylinder is \((r_0 + r \cos \phi, r \sin \phi, z)\). Therefore

\[
\frac{r_0^2 + r^2 + z^2}{z_1^2 + \sqrt{r_0^2 + r^2 + z_2^2} + 2r_0 r \cos \phi} \quad (A11)\]

which requires Gaussian quadrature.

The solid angle of a cylinder is equal to the negative of the sum of the solid angle of the open ends. Hence, Eq. A7 is applied to each end for computation of \((H)_{ij}\).

**Transition elements**

When triangular discretization meets cylindrical discretization, there must be a transition element. The triangular discretization will never be able to produce an exactly circular opening by using straight line segments resulting in flux leakage if a perfectly cylindrical element were to be attached. Instead, an element with aalong one axis, the solid angle can be expressed in terms of

\[\cos 2\alpha = \frac{r_{AS} \cdot r_{AE}}{|r_{AS}| |r_{AE}|} \quad \text{and} \quad \cos 2\beta = \frac{r_{AS} \cdot r_{AE}}{|r_{AE}|^2}\]

**FIG. A2.** Computing solid angle of a disk of radius \( \rho \) (shaded region) is simplified if disk is considered a cross section of a right elliptical cone. Dashed line, central axis of cone.
the element is treated as a cylinder but for bipolar calculations, the negative of the solid angles of the disk on one end and the triangles on the other end is used. Hence, through use of this transition element, gaps in the surface are avoided that would invalidate the BEM.

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