Macrosopic and Subcellular Factors Shaping Population Spikes


Macroscopic and subcellular factors shaping population spikes. J. Neurophysiol. 83: 2192–2208, 2000. Population spikes (PS) are built by the extracellular summation of action currents during synchronous action potential (AP) firing. In the hippocampal CA1, active dendritic invasion of APs ensures mixed contribution of somatic and dendritic currents to any extracellular location. We investigated the macroscopic and subcellular factors shaping the antidromic PS by fitting its spatiotemporal map with a multineuronal CA1 model in a volume conductor. Decreased summation by temporal scatter of APs reduced less than expected the PS peak in the stratum pyramidale (st. pyr.) but strongly increased the relative contribution of far dendritic currents. Increasing the number of firing cells also augmented the relative dendritic contribution to the somatic PS, an effect caused by the different waveform of somatic and dendritic unitary transmembrane currents ($I_{\text{m})}$. Those from somata are short-lasting and spiky, having smaller temporal summation than those from dendrites, which are smoother and longer. The different shape of compartmental $I_{\text{m}}$ is imposed by the fitting of backpropagating APs, which are large and fast at the soma and smaller and longer in dendrites. The maximum sodium conductance ($g_{\text{Na})}$ strongly affects the unitary APs at the soma, but barely the PS at the stratum pyramidale (st. pyr.). This occurred because somatic $I_{\text{Na}}$ saturated at low $R_{\text{Na}}$ due to the strong reduction of driving force during somatic APs, limiting the current contribution to the extracellular space. On the contrary, $R_{\text{Na}}$ effectively defined the PS amplitude in the st. radiatum. The relative contribution of dendritic currents to the st. pyr. increases during the time span of the PS, from $\sim30–40\%$ at the peak up to $100\%$ at its end, a pattern resultant from the timing of active inward currents along the somatodendritic axis, which delay during backpropagation. Extreme changes imposed on dendritic currents caused only moderate effects on the st. pyr. due to reciprocal shunting of active soma and dendrites that partially counterbalance the net amount of instant current. The amplitude of the PS follows an inverse relation to the internal resistance ($R_i$), which turned out to be a most critical factor. Low $R_i$ facilitated the spread of APs into dendrites and accelerated their speed, increasing temporal overlapping of inward currents along the somatodendritic axis and yielding the best PS reproductions. Model reconstruction of field potentials is a powerful tool to understand the interactions between different levels of complexity. The potential use of this approach to restrain the variability of some experimental measurements is discussed.

INTRODUCTION

During synchronous activation of regularly arranged neuron ensembles, the coactivated transmembrane currents ($I_{\text{m})}$ add in the interstitium rising characteristic field potentials (FPs). Because of their reproducibility and stability they are widely used to study the global response of neuron nuclei plus the average behavior of individual neurons and subcellular electrical events. A precise correlation between field and subcellular events is hindered because the extracellular part of the $I_{\text{m}}$ spread within the tissue in all directions, so that FPs always are contributed by currents arising from different cell domains. These so-called volume-propagated currents are inherent to extracellular recordings. However, the theoretical knowledge relating extracellular currents and field potentials is well developed (Lorente de Nó 1947a; Nicholson 1973) and allows the identification of subcellular membrane generators during synchronous activation of architectonically complex neuron ensembles.

Our initial goal in this study was to correlate the averaged electrical responses of homogeneous populations of neurons with the structural and biophysical findings obtained with modern techniques at the subcellular level. These have revealed a variety of voltage-gated channels in distinct subcellular domains (Hoffman et al. 1997; Magee and Johnston 1995a,b) that necessarily will be coactivated in variable degrees during neuronal operation, their interplay being essential for neuronal integration. We have extrapolated the available empiric data using a realistic multineuronal model of the highly laminated CA1 region to reconstruct the simplest spatiotemporal FP map that is obtained during synchronous antidromic action potential (AP) firing, i.e., the population spike (PS). It can be anticipated that the small errors on subcellular parameters that would not affect notably the physiology of a single-cell model, however, may originate large deviations from the experimental results when accumulated to reproduce the PS. The process of minimizing these divergences constitute a powerful tool to study the critical factors involved in shaping evoked FPs (Klee and Rall 1977; Rall and Shepherd 1968) and to restrict the parametric space of single-cell models.

The customary hippocampal PS is considered a reliable index for the number of synchronously firing neurons (Andersen et al. 1971) and so has been used to test changes in average neuron excitability in countless studies of physiological phenomena, such as synaptic plasticity. A critical consideration of its extracellular nature is missing, however. The mentioned interpretation requires that the current contributed by each neuron along its entire morphology remains constant in different physiological situations, an assumption hard to reconcile with some major experimental observations. Thus APs are fired in the axon and soma membranes, but they can actively spread to or even be initiated in dendritic regions of many neuronal types (Chen et al. 1997; Jefferys 1979; Larkum et al. 1996; Regehr et al. 1992; Turner et al. 1991; Wong et al. 1979). This being long known for CA1 pyramidal cells from...
the inspection of PS profiles and current-source density (CSD) analysis (Fujita and Sakata 1962; Herreras 1990; Leung 1978; Sperli et al. 1966), the relative contribution of the spatially segregated somatic and dendritic action currents to the PS has been rarely considered (e.g., Lorente de Nó 1947b). In fact, we recently have demonstrated that backpropagating dendritic APs may contribute as much as 40% to the antidromic PS amplitude recorded in the stratum pyramidale (st. pyr.) in vivo (López-Aguado et al. 2000). Because somatodendritic action currents can be modulated locally (Callaway and Ross 1995; Herreras and Somjen 1993; Mackenzie and Murphy 1998; Spruston et al. 1995), a further interest of this study is to obtain some clues as to how the electrical activity of a particular neuronal domain (soma, dendrites or axon) contributes to shape the FPs recorded in regions spatially occupied by or shared with others.

Specifically, we sought to determine the effect of the asynchrony of activation, the cell number (aggregate size), and the relative contribution of different cellular domains shaping the PS. As a necessary step, the initiation and propagation of APs and the passive and active properties of individual cells that give them shape, including channel distribution and kinetics, also were correlated. Some of the results have been presented in preliminary form (Herreras et al. 1997; Varona et al. 1998).

Methods

Experimental Procedures

General. Female Sprague-Dawley rats weighing 200–250 g were anesthetized with urethan (1.2–1.5 g/kg ip) and fastened to a stereotaxic device. The animals were breathing spontaneously. Heart rate was monitored continuously, and body temperature was kept constant at 37 ± 0.1°C with a heating blanket. Surgical and stereotaxic procedures were as previously described (Herreras 1990; Herreras et al. 1994). Two concentric bipolar stimulating electrodes were positioned in the alvear region and in the ipsilateral CA3 for antidromic and orthodromic activation of the CA1 pyramidal population, respectively (0.07–0.1-ms square pulses, 0.1–0.5 mA). A subcutaneous Ag/AgCl wire electrode under the neck skin was used as reference. The recording electrodes were connected to DC-coupled field effect transistor (FET) input stages. The characteristic configuration of evoked potentials guided the placement of the recording electrodes (Herreras 1990).

EXTRACELLULAR RECORDING AND CSD ANALYSIS. Recording electrodes were glass micropipettes backfilled with 150 mM NaCl (3–6 MΩ). One micropipette remained stationary in the CA1 somatic layer throughout the experiment in order to test the constancy of the evoked PS amplitude, and another micropipette was used to explore dorsoventral trajectories in 25- or 50-μm steps driven by a piezoelectric micromanipulator. After filtering (1 Hz to 5 kHz band-pass) and amplification, signals were recorded on VCR, acquired to a computer (20- to 40-kHz acquisition rate, Digidata 1200, Axon Instruments, Burlingame, CA) and processed by Axotape software (Axon Instruments), and then further analyzed by the Axum program (Trimetrix, Seattle, WA). Depth profiles of evoked FPs were used for CSD analysis, which provides the magnitude and location of the net \( I_n \) generated by unclamped neuronal elements contained within a very small portion of tissue. A detailed account of technical and theoretical considerations for the calculation of CSD in vivo has been presented elsewhere (Herreras 1990). We have assumed that the extracellular space is homogeneous and isotropic, the derived errors can be ignored (see following text). Using the method by Demirci et al. (1997), we measured an average \( R_p \) of 300 Ω·cm across the CA1. The customary unidimensional approach for the calculation of \( I_{CSD} \) in the main cell axis \( z \) was approximated by the following equation (Freeman and Nicholson 1975)

\[
I_{CSD} = -\Phi(z + h) - 2\Phi(z) + \Phi(z - h)
\]

where \( \Phi \) is the field potential and \( h \) is the distance between two consecutive recording points. Smoothing procedures aiming to decrease high spatial noise were not used, for they introduce important perturbations in the relative amplitude and spatial distribution of high-frequency components, i.e., action currents (Herreras 1990). The required condition of homogeneous activation was satisfied by recording from the maximally activated lamella. The high sensitivity of the fast transients of evoked potentials to mechanical interference is a major source for perturbations on the calculated CSD profile (Herreras 1990). Then after each movement of the exploring pipette, recording did not begin until response stabilized. Also because of marked changes in hippocampal excitability during different electroencephalographic (EEG) patterns (Herreras et al. 1987), the collection of data was performed only while the hippocampus displayed the typical slow irregular activity.

INTRACELLULAR STUDY. Micropipettes (1.5 mm OD) were back-filled with 2–4 M potassium acetate (60–120 MΩ). Signals were amplified using a bridge circuit amplifier, filtered at 10 kHz, and stored on VCR for later analysis by using pClamp and Axotape computer programs (20- to 40-kHz acquisition rate). With the skull fastened to the stereotaxic device, brain pulsations were greatly reduced by lifting the rear quarters of the animal and, on occasions, the cisterna magna also was drained out. Care was taken to avoid the overlying cortex from air exposure. No further requirements were needed to obtain stable lasting impalements (≤96 min). Recordings were made from electrophysiologically identified pyramidal cells located in the st. pyr. of the CA1, identified by the characteristic field potential. After impalement of a cell, I-V plots were obtained every 10 min to ensure cell stability. Cells that did not fire a single antidromic AP after alvear stimulation were rejected. We have considered healthy cells those having resting membrane potential more negative than −60 mV (−67.6 ± 2.2 mV; \( n = 16 \)) and overshooting APs. The average apparent input resistance calculated from the linear range of the I-V plots was 31.2 ± 2.7 MΩ (range, 18–42) which is within reported values for sharp electrode recordings (e.g., Turner et al. 1991). Once stabilized, most cells fired spontaneous APs at a very low rate (<1 Hz).

Computer Model

ARCHITECTONIC ORGANIZATION OF THE AGGREGATE. The dorsal CA1 region was modeled with aggregates of different size preserving an experimentally observed cell density of 64 neurons oriented in parallel in a 50 × 50 μm anterolateral lattice (Boss et al. 1987). The anteroposterior and lateromedial dimensions of the aggregates were 0.05 × 0.05, 0.2 × 0.2, 1 × 0.35, 1 × 1, and 3 × 2 mm, corresponding to 64, 1,024, 6,072, 17,424, and 104,544 morphologically identical model neurons in the total volume, respectively. The dimensions and cell number of the largest aggregate can be taken as a rough estimation of actual values for the dorsal CA1 region. Dorsoventral extension was set to 0.8 mm (from the alveus to the distal apical tuft). Three different spatial distributions of neurons were analyzed with the st. pyr. (50-μm thick) containing their somata and arranged either in a monolayer, four layers of even density, or a realistic distribution of four uneven layers with 66% of somata in the apical side and 22 and 11% in the two layers of the basal side (proportions are rough estimations made on our previous histological material; see schemes in Fig. 3). Each neuron was rotated a random angle around the somatodendritic axis to ensure that the particular three-dimensional (3-D) morphology used in our experiments introduced no artifacts in the \( \Phi(t) \) calculations. The slight curvatures of the dorsal CA1 region were neglected for this study.
CALCULATION OF THE MODEL FIELD POTENTIAL. A set of 16 “recording” points 50 μm apart simulating a vertical track spanning from 250 μm above to 500 μm below the st. pyr. was placed at the center of the population in parallel to the somatodendritic axis (we termed as b5-b1, s and ap1-ap10 levels, to designate the successive positions at the basal tree, st. pyr. and apical tree, respectively). The value of the FP measured at each point was calculated as follows

\[ \Phi(t) = \frac{1}{4\pi} \sum_{i=1}^{\text{cells}} \sum_{j=1}^{\text{comps}} \frac{I_{m}(t)}{r_{ij}} \]

where \( I_{m} \) is the total membrane current at the \( i \)th compartment of neuron \( i \), and \( r_{ij} \) is the distance from the recording point to that compartment. Thus compartments are treated as point sources into a medium of homogeneous conductivity. The current density was obtained as in the experimental CSD analysis (see preceding text). Compartmental transmembrane currents were calculated using the GENESIS simulator (Bower and Beeman 1998). Calculations of FPs and CSDs were programmed in C code. For a thorough model description see http://navier.ucsd.edu/ca1ps.

NEURON MODEL PROTOTYPE. The single-neuron model reproduced the detailed pyramidal cell morphology, with an average dendritic branching pattern, total dendritic length, dendritic tapering, and distribution of spine density obtained from detailed morphometric studies (Bannister and Larkman 1995a,b; Trommald et al. 1995) (see: http://navier.ucsd.edu/ca1ps for details). The 3-D morphology was simulated using 265 compartments, distributed in an axon, [consisting of myelinated portions, Ranvier nodes, initial segment (IS) and axon hillock (AH)], soma, and apical and basal dendritic trees [a two-dimensional (2-D) projection of the model neuron is shown in Fig. 2]. Further partitioning of the neuron was unnecessary for the current purposes. Compartment length was always \( >0.01 \) and \( \text{<0.2} \lambda \). Spatial coordinates for each compartment can be found in the preceding http address. Total effective area of the neuron was 66,800 μm\(^2\) (including spine area). The electrotonic parameters for the majority of the simulations were \( R_{m} = 70,000 \Omega \cdot \text{cm}^{-2} \), \( R_{i} = 150 \Omega \cdot \text{cm} \) (but see Fig. 8), \( C_{m} = 0.75 \mu \text{F}/\text{cm}^{2} \). Values of \( R_{m} \) and \( C_{m} \) at the dendritic compartments were compensated to take into account spine area. The input resistance measured at the soma was 140 MΩ, and \( \tau \) was 25 ms, values between those reported for whole cell recordings (e.g., Spruston and Johnston 1992). We checked the effect of a somatic shunt to reproduce the leak caused by sharp electrode penetration (up to an input resistance of 45 MΩ) and found a negligible decrease of the model PS (\( \sim \%1 \)).

We used seven ionic channels to simulate the active properties of the somatodendritic membrane: fast sodium (Na\(^{+}\)), calcium (Ca\(^{2+}\)), and five potassium currents: delayed rectifier (DR), small persistent muscarinic (M), A-type transient (A), short-duration \([\text{Ca}]\)- and voltage dependent (C) and long-duration (C)-dependent (AHP). Conductance variables were described with Hodgkin-Huxley type formalism. Because of lengthy description of kinetics, we only provide here the sources and modifications (see http address in the preceding text for details). Except when specified, the kinetics of all these channels were obtained from Warman et al. (1994) with the following modifications: the activation time constant for the Na\(^{+}\) channel was still halved due to the faster rising slope of in vivo APs (see RESULTS); for the Ca\(^{2+}\) channel, a temperature of 35°C was used in the Nernst equation, \( E_{\text{Ca}} = -13.275 \text{mV} \cdot [\text{Ca}]/[\text{Ca}_{i}] \), where \( [\text{Ca}_{i}] = 1.2 \text{ mM and basal [Ca] is 50 nM} \); for the C-type K\(^{+}\) channel, \( \alpha_{c} = -0.0077 \cdot (V_{m} + V_{\text{shift}} + 103) \cdot \exp[(V_{m} + V_{\text{shift}} + 103)/-12] - 1 \). As described by Warman et al. (1994) and Bannister and Larkman (1995a,b), \( [\text{Ca}_{i}] \) was simulated as two different Ca pools, with \( \tau = 0.9 \text{ ms for the calculation of } E_{\text{Ca}} \) and modulating the C-type K\(^{+}\) current, and \( \tau = 1 \text{ s for the AHP-type K}^{+} \text{ current.} \)

For the neuron prototype in Fig. 7, we used an A-type K\(^{+}\) channel modified from Hoffman et al. (1997): \( I_{A} = g_{A} \cdot a^{b} \cdot (V_{m} - E_{K}) \), \( \tau_{a} = 0.15 \text{ ms, } E_{\text{Ca}} = 1/(1 + \exp(-5 V_{m}/10)) \) for proximal dendrites (\( \pm 150 \mu \text{m from the soma} \)), and \( E_{\text{Ca}} = 1/(1 + \exp(-15 V_{m}/8)) \) for distal dendrites, \( \tau_{a} = 5 \text{ ms if } V_{m} < -30 \text{ mV}\), \( \tau_{a} = 5 + 0.26(V_{m} + 30) \) if \( V_{m} \geq -30 \text{ mV} \). \( E_{K} = 1/(1 + \exp(-56 V_{m}/-8)) \). In the axon, Na\(^{+}\) channels were identical as for the somatodendritic membrane, whereas the DR-type K\(^{+}\) channel was obtained from Traub et al. (1994). Equilibrium potentials were +45 and −85 mV for Na\(^{+}\) and K\(^{+}\), respectively. Because of the objective of this work requires the simultaneous reproduction of unitary APs and the aggregate PS profile, the channel distributions along the cell morphology have been tuned continuously in a feedback manner. The detailed distributions for the neuron prototypes shown in Fig. 7 can be obtained from http://navier.ucsd.edu/ca1ps. Simulation of antidromic stimuli was made by 1-na, 0.1-ms long pulses in a Ranvier node for each cell in the population. Alpha functions were used to simulate synaptic activation as follows: \( g_{\text{syn}}(t) = \frac{g_{\text{syn}}(t)}{(\tau_{\text{syn}})} \{\exp(1 - t/\tau_{\text{syn}})\} \). The synaptic currents are defined as \( I_{\text{syn}} = g_{\text{syn}}(t) \cdot (V_{m} - E_{\text{syn}}) \), with \( \tau_{\text{syn}} \) of 7 ms and reversal potential of −75 mV for GABA\(_{A}\) mediated currents. These were distributed in the soma and proximal apical shaft, and initiated 1.5 ms after antidromic activation.

Comparisons between experimental and model potentials: the relevant parameters

For a fully safe interpretation of the experimental PS based on model reconstruction, an exact fitting would be required, and the comparison of the entire spatiotemporal PS map should be performed. Statistical global comparisons are difficult and may lose physiological meaning due to the different weight of components. In practice, we found more useful a direct comparison of the parameters that can be measured easily by experimentation. At the single-cell level, we used the amplitude, half-width, and rates of rise and fall of the AP measured at the soma. Reliable AP parameters at dendritic levels in vivo are not available, thus an initial approximation was made using the in vitro AP estimates (Spruston et al. 1995; Turner et al. 1991) and later modified as required once the FP and L\(_{\text{CSD}}\) maps have been computed. At the aggregate level we measured the amplitude, width, and latency of the PS, and the magnitude and duration of the L\(_{\text{CSD}}\) at the st. pyr. and stratum radiatum (st. rad.) Experimental values are given as the means ± SE.

Although obvious, we want to emphasize that fitting of partial data can be achieved by many different combinations of the large number of parameters used in the model. The experimental estimations for subcellular parameters, which constitute the basic elements for the computations, are far less reliable than the unitary AP parameters, and so are these when compared with the highly steady PS. In this study, fitting the unitary AP parameters constitutes a crucial intermediate step and an important advantage, as it strongly defines the magnitude and time course of the compartmental \( I_{m} \) necessary for FP computation. This process yields a strong reduction of the suitable combinations of subcellular parameters that will be limited further after the ensemble FP is computed and compared with the experimental one. For the purposes of the present study, the mentioned reference parameters are satisfactory. The fitting of realistic AP waveforms to obtain the \( I_{m} \) analogous to the use of AP waveforms as the voltage command in voltage-clamp experiments. In a way, the model is more realistic because the voltage command is specific for each membrane subregion, which cannot be achieved in actual experiments. This is an important advantage, for activated adjacent membranes act as reciprocal shunts, shaping each other’s \( I_{m} \) (see López-Aguado et al. 2000) (see also RESULTS).

Sources of error

The lack of precise experimental values for some parameters is a potential source of error. The electrotonic parameters, some are worth mentioning for their relevance. Our own preliminary mea-
measurements of $R_g$ in vivo indicate that the st. pyr. is about twice that in dendrites. It is known that this anisotropy causes an overestimation of st. pyr. currents that, however, does not modify their estimated location (Holsheimer 1987; Okada et al. 1994). In our study, this led to an underestimation of the FP amplitude at the st. pyr. as compared with dendritic locations. Uncertainty exists also for the value of $R_g$, the used values specified and justified in RESULTS. The available data on channel kinetics, maximum conductances, and their spatial distributions are partial and far from reliable for the accuracy required in this study. However, fitting the realistic AP parameters along the somatodendritic axis as an intermediate step provides values for channel kinetics that can be examined and compared with those experimentally obtained and also produces the spatial distribution and the magnitude of the underlying $I_m$, which are the only important variables for the calculation of the model FP. Finally, a deficiency of the model is that the calculation of $V_m$ does not take in account the ongoing variation of FP. On one side, including this feature in the model would imply a large computational cost, and on the other side, only slight quantitative but no qualitative changes should be expected. A different source of error arises from anatomic and morphological considerations. The use of a single tridimensional morphology for the prototype model cells leads to an unrealistic spatial clustering of identical dendritic portions. It is known that the apical shaft has variable length and that the branching pattern varies accordingly (Bannister and Larkman 1995a). Presumably, using multiple morphologies will cause some spatial averaging of ensemble dendritic currents. Also we found that the fanning angle of dendritic trees has some impact on volume propagated currents (halving and doubling the used fanning angle caused the antidromic PS to vary $\pm 10\%$).

RESULTS

General features of real and model pyramidal neurons

Intracellularly recorded pyramidal cells fired a single AP followed by a fast (presumably GABA$_A$) inhibitory postsynaptic potential (IPSP) or an AP riding on an excitatory postsynaptic potential (EPSP) and followed by a fast and a slow (presumably GABA$_B$) IPSP after anti- or orthodicritic activation, respectively (Fig. 1, top). They also behaved as expected to current injections, displaying fast and slow intraintrain accommodation and slow afterhyperpolarization (Fig. 1, bottom). All prototype model neurons were adjusted so as to reproduce these general features (an example is shown in Fig. 1), although a fine adjustment is unnecessary at this stage for they are barely relevant for the antidromic PS. Of critical relevance, however, are the fine details of the AP and its subcellular peculiarities governed by the fast Na$^+$ and K$^+$ channels. These will be studied in the following text in relation to their impact on the model PSs.

Experimental antidromic PS: subcellular localization of current generators

Maximal stimulation of the posterior alveus triggered synchronous APs originating a characteristic extracellular sharp monophasic negative spike (i.e., the PS; amplitude: 24.96 $\pm$ 0.51 mV; half-width: 0.63 $\pm$ 0.01 ms) at the level of the st. pyr. (thick trace in Fig. 2A, FP) that became a positive-negative biphasic spike for $\sim 200$–$250$ $\mu$m within the proximal stratum radiatum (st. rad.), and a pure positive spike at more distal positions as the negative component gradually faded. The PS recorded immediately below the st. pyr. was up to 2–3 mV larger. Within the stratum oriens (st. or.), the PS unfolded in a two-spike sequence toward the alveus, the first (asterisk) remaining stationary, whereas the second (dot) increased in latency and declined faster.

In CSD analysis, extracellular sinks and sources correspond to net cellular inward and outward currents, respectively, terms that will be handled as synonymous throughout the text. The earliest current was a pure sink (net inward currents during AP) at the level of the st. pyr. (Fig. 2A, right), where it reached maximal amplitude ($2.09 \pm 0.11$ A/cm$^2$). The propagation of this sink into the apical and basal dendritic trees (Fig. 2A, arrows) indicated active dendritic invasion of the AP for $\sim 225$ and 125 $\mu$m, respectively. Except in the st. pyr., the sink always was preceded by a passive source (Fig. 2C) corresponding to the leading outward passive currents (capacitive and leak) closing loop with the active cell domains. The propagating source/sink sequences account for the second negative spike (dot) and its preceding positive interlude in the st. or. (Fig. 2B, double-headed arrow), and the biphasic spike in st. rad. A decreasing source of constant latency was observed distally as expected in the region where the AP propagation became passive. This source corresponds to capacitive outward currents, although active K$^+$ currents also may contribute. Net inward currents in dendrites were always smaller but lasted longer than in the st. pyr. (Fig. 2C). The shorter duration in st. pyr. may be caused by a more spiky local $I_m$ of the cell somata, and/or the longer duration in dendrites may be due to spatial averaging of multiple secondary dendritic branches activated.
with different delays. Measured from its origin at the summit of the preceding source, the peak amplitude at each of the three successive stages below the st. pyr. (in 50-$\mu$m steps) was $1.4 \pm 0.09$, $0.98 \pm 0.09$, and $1.22 \pm 0.1$ A/cm$^2$, respectively, decreasing thereafter to extinction. The speed of the dendritic backpropagation was faster in the apical than the basal dendritic tree and slower the farther from cell somata, as noted on the leading outward currents that began progressively more delayed (Fig. 2C). Using the latencies at their summit (Fig. 2C, small vertical bars), we measured an average speed of $0.47 \pm 0.02$ m/s along the apical shaft (from 50 to 150 $\mu$m below the st. pyr.).

For the model, it was important to gain quantitative information on the contribution of IS/AH currents, as they are possible strong current generators. Using a 25-$\mu$m spatial resolution, the initial sink began simultaneously and without leading sources in the basal and apical halves of the st. pyr., but closer to the st. or. border, where ISs membranes dominate (sp1 in Fig. 2C), the sink was smaller and began slightly earlier. We did not find a spatially segregated current sink attributable to IS/AH currents. However, the notch at the lowering limb of the somatic sink (arrow in sp1, Fig. 2C) likely results from a slight delay on the sequential activation of two partially overlapped generators, the IS and the soma/basal stems, the first of which gave rise to the first stationary negative peak of the FP in the st. or. This peak also was shaped on its rising limb by the leading passive sources of the colocalized significant fraction of basal stems, as they are sequentially invaded by the AP (Fig. 2B, double-headed arrow). We recently reported the unmasking of the first negative peak during selective blockade of the second one that marks the AP basal invasion (López-Aguado et al. 2000). In any case, the net contribution of the ISs currents is small and should be so reproduced in the aggregate model. In contrast, we were able to discriminate, in some experiments, a small source/sink sequence (Fig. 2B) located at $\sim 200$–$250$ $\mu$m above the st. pyr. that clearly initiated earlier than any sink at the st. pyr. (small arrows in Fig. 2B, $I_{\text{CSD}}$), most likely caused by synchronous APs at the clustered Ranvier nodes in the alveus, which admittedly contain a very high density of Na$^+$ channels. This sink contributes to the initial part of the first negative peak (arrow in Fig. 2B, FP).
CA1 model antidromic PS

Every combination of the main macroscopic factors, cell number, spatial arrangement, and asynchrony of activation, has been analyzed, and we show only those that accentuate individual effects. In later sections, it will be shown that the effect of some of these factors on the FP may be obscured or potentiated at specific values of the others, but it is not qualitatively modified by the use of other channel distributions in the component model cells. Thus a good fit of model to experimental PS is not essential at this stage.

RELATION TO THE NUMBER OF FIRING CELLS. We searched for the minimum number of cells required to reproduce a maximal antidromic PS with CA1 aggregates of increasing size and constant cell density (see METHODS). When each cell in the aggregate was activated antidromically without temporal scatter, the antidromic PS amplitude at the st. pyr. and proximal apical dendrites (ap1-ap3) was near saturation for a strip 2 mm wide and 3 mm long, corresponding to a number of cells roughly similar to actual estimations for the dorsal CA1 region, i.e., ~10^5 pyramids (Boss et al. 1987). This is patent from the corresponding curves shown in Fig. 3A where the PS has been plotted as a percentage of the maximum amplitude at each recording level for the largest aggregate. The more distal was the recording point, the faster was the rate of the PS increase with the cell number. It became clear from the shape of the distal plots that even with the largest aggregate the PS is far from saturation. Therefore using aggregates smaller than real causes a variable underestimation of the PS amplitude at different strata. The differential saturation occurred because the uneven quantitative distribution of net currents along the somatodendritic axis (Fig. 2C), allowing volume-propagated currents to reshape FPs. The contribution of far neurons to a distant electrode, though weighed by distance, is large so that the relative contribution of nearer generators is decreased. In consequence, the PS recorded in strata dominated by weakly excitable cell subregions, such as distal dendrites, has stronger proportional contribution of volume-propagated currents and is more sensitive to the aggregate size. By the same token, in aggregates of equal size, the less active are modeled the distal dendrites the higher is the underestimation for their calculated

FIG. 3. Effect of the aggregate size, latency variability of activation and somata distribution on the model PS (FP) and the ensemble local currents (I_{CSD}) during antidromic activation. A: amplitude of the antidromic PS recorded from the soma layer (s) to the last apical level (ap10, 500 μm below) is plotted against the cell number after normalization with the value obtained for the largest aggregate (~10^5 cells). All simulations shown in this figure employed a neuron prototype with active apical dendrites that enabled AP backpropagation. Varying saturation of the PS amplitude is evident at different recording levels. The smaller the aggregate size and the weaker the current generators, the larger is the underestimation of the PS amplitude. Arrow points to the aggregate size used in all other figures. Latency variability: none. Somata distribution: even (see D). B: estimation of the density of currents for cubes of tissue (50 μm side) centered at the indicated levels of recording. Note the similitude of the model waveforms with those obtained from actual recordings (Fig. 1C). Latency variability: none. Somata distribution: even (see D). C: increasing the temporal scatter of activation among the neurons of the aggregate caused a notable decrease of the ensemble currents (I_{CSD}) in regions dominated by neuronal elements generating short-lasting currents (soma layer, s) due to poor temporal summation in the extracellular space, whereas the effect was less notable in apical dendrites. Only unrealistic dispersion (1 ms on average) of the AP latency had a notable impact on the antidromic PS at any level. Somata distribution: even. Aggregate size: 17,424. D: the effect of spatial cancellation of currents from different neuronal elements within the soma layer (initial segments, somata and dendrites) was analyzed by using different arrangements of the cells with their somata distributed in a monolayer, 4 layers of homogeneous density (even), or the realistic uneven distribution with 66% of the somata accumulated in the apical side of the stratum pyramidale (see RESULTS for explanation). Latency variability: none. Aggregate size: 17,424.
FP. To gain computer efficiency, all data presented in the following text were obtained using an aggregate of 17,000 pyramids (1 × 1 mm). This constitutes an underestimation <10% for the regions where we focused our study between the st. pyr. and 150 μm below cell somata (to ap3 levels).

The net currents underlying the model FP profiles were calculated as for in vivo. The waveforms of I_{CSD} are roughly similar to those experimentally obtained (compare waveforms in Figs. 2C and 3B), displaying the largest sink at the st. pyr. and source-sink sequences along the apical tree. The spiky waveform of the sink at the st. pyr. matched the waveform of the single cell I_m at the soma (e.g., Fig. 4A), an expected result in absence of latency variability among the activated cells. Except for very small aggregates, the I_{CSD} profile was the same regardless of the aggregate size because currents generated by neuron elements out of the considered volume are excluded by CSD analysis. Thus the net contribution of neurons within tissue spheres concentric to the electrode can be estimated. In the example of Fig. 3A, 30% of the PS amplitude at the st. pyr. is contributed by the neurons located 100 μm away from the electrode. It must be reminded that volume-propagated currents to a distant electrode arise from different subcellular domains, equalizing the FPs across different strata that are so less reliably interpreted as identifying local subcellular events (note discrepancies between polarity of FPs and I_{CSD} in Fig. 2A).

**EFFECT OF THE ASYNCHRONY OF ACTIVATION.** A small variability in the latency of antidromic activation of individual cells is known to occur (Lipsky 1981). The effect of this small asynchrony or temporal scatter on the PS was studied by activating each cell with a random delay of variable dispersion ~1 ms (roughly the reported experimental range) (see Andersen et al. 1971; Herreras et al. 1987) obtained from a binomial distribution. In general, the temporal scatter affects more markedly I_{CSD} than FPs (Fig. 3C). The longer the temporal scatter the smaller and rounded the resultant FP and I_{CSD}, but the effect was only meaningful in cell domains where net local currents are short lasting and spiky, as the soma and axon membranes. A temporal scatter of 0.25–0.5 ms, roughly approaching that experimentally obtained (Andersen et al. 1971), caused notable changes in the initial PS slope at the st. pyr., but it barely affected its amplitude. The differential effect of asynchrony on somatic I_{CSD} and FPs may appear paradoxical. In fact, it is caused by the interaction of asynchrony with some other subcellular factors that determined different waveforms of compartmental currents (see following text).

**EFFECT OF THE SPATIAL OVERLAP OF DIFFERENT SUBCELLULAR GENERATORS.** The spatial overlap of different cell generators is only relevant at the st. pyr., where ISs, and basal and apical dendritic stems are mixed with the dominant somatic generators. We studied the effect of this overlap by using three different distributions of cell somata, monolayer, four-layered of even density, and the realistic uneven distribution (Fig. 3D), while maintaining cell density. Largest FPs and I_{CSD} were obtained in the monolayer configuration only in the st. pyr. (~20%), as expected in absence of spatial scatter for the strongest generators (somata). Minor differences were noticed between the even and uneven arrangements, the latter showing a slight shift of the maximum PS amplitude toward the apical side, as also found in experiment. This result persisted even with neuron prototypes designed with large differences in soma and IS channel densities. Again, in smaller aggregates the effects were much more marked because the smaller equalizing influence of far located currents.

![FIG. 4. Different values of somatic g_{Na} optimize antidromic AP features and antidromic PS amplitude in large cell aggregates. A: linear increase of somatic g_{Na} from 180 to 1,080 pS/μm² increases the local I_m peak (arrow) in individual cells, and the AP rising slope, although the amplitude saturates rapidly. The lower the g_{Na}, the more delayed is the AP invasion from the axon and an AB brake appears (arrowhead). B: using large neuron aggregates (n = 17,424) and a temporal scatter (t.s.) of 0.25 ms (top), the peak of ensemble currents (I_{CSD}) at the st. pyr. rapidly saturated due to poor temporal summation of the short-lasting individual I_m peaks, and the corresponding PSs behaved similarly (horizontal arrows). By repeating the computations without temporal dispersion (bottom), the somatic peaks are locked in phase in the extracellular space, facilitating their summation, and the ensemble currents grow accordingly as expected from the increase of individual somatic I_m (curved arrow). Unexpectedly, the corresponding PSs reached maximal amplitude at low g_{Na} (horizontal arrow). However, 2 minima are disclosed, an initial rapid peak that matches in time of that of the ensemble somatic currents (arrowhead), and a subsequent peak on a slower component (small arrow). Later hump must be due to volume propagated dendritic currents not discernible on the t_s plots, which only reflect local somatic currents. This later peak has a larger absolute value than the first. Apparently, the PS amplitude is weakly dependent on the somatic g_{Na} of individual neurons.](http://jn.physiology.org/)

**Sodium channel density differentially affects the single-cell AP and the ensemble field potentials at the somatic region**

The PS amplitude depends on the instant amount of extracellular current during synchronous firing of APs, and these are dominated by the inward I_{Na}. Thus we checked the effect of somatic g_{Na} density at the soma of a neuron prototype with a moderately excitable apical tree that enabled active AP back-propagation. The obligatory restriction to the useful range of g_{Na} is that the APs of the component model neurons must fit the values of the experimental APs. Because notable differences have been reported in in vitro experiments, we used the values of APs recorded in vivo. Evoked APs were always smaller (~20 mV) than spontaneous APs, often even after calculating the actual V_m by subtraction of the extracellular PS
(Fig. 1, top) obtained on withdrawal of the pipette from the cell. By using the $V_m$, we found that the apparent threshold, rate of rise, and half-width for spontaneous and evoked APs varied slightly; then we used the parameters of the former to prevent errors in the calculation of $V_m$. These always were preceded by a slow depolarizing ramp (threshold: 16.5 ± 1.1 mV), had a rate of rise of 399.1 ± 26.9 V/s (range 232–640), measured as the 10–80% slope from threshold to peak) and a falling rate of 103 ± 3 V/s. The AP amplitude from rest was 90.7 ± 1.9 mV (range 83–104), and the half-width was 0.82 ± 0.01 ms. An AB-brake was not distinguishable on the rising limb, usually not even in differentiated records. This can only be disclosed under conditions of decreased IS/AH-to-soma safety factor for invasion, e.g., during high-frequency antidromic activation (López-Aguado et al. 2000).

Using a model neuron with soma $g_{Na}$ varying from 180 to 1,080 pS/μm², the somatic AP grew from 83 to 102 mV and the rising slope from 192 to 688 V/s (Fig. 4A, $V_m$). The average experimental APs were better fitted by a $g_{Na}$ density of ~500 pS/μm², whereas those in the upper range required ~900 pS/μm². In the model, the lowest value yielded APs with very slow rising slope and disclosed a distinct AB-brake (arrowhead). The somatic $I_m$ (Fig. 4A, $I_{CSD}$) presented a fast peak declining to a plateau-like current, smaller the higher the $g_{Na}$. The peak increased from 3.2 to 6.5 nA through the specified $g_{Na}$ range. It is important to note that because the increment of $I_{CSD}$ at the IS did not result in larger $I_m$ (Fig. 4A). Changes of $g_{Na}$ at the IS did not modify the somatic $I_m$ noticeably.

During aggregate activation, the temporal scatter of activation and the added volume-propagated currents from other cell generators (mostly dendrites), made the waveform of the PS at the st. pyr. to differ from the single-cell somatic $I_m$ (Fig. 4B, FP, top). We might expect that increasing $g_{Na}$ will cause a parallel increase of the antidromic PS amplitude because FP s depend on $I_m$, but it saturated at low $g_{Na}$ densities. Even more striking was that the corresponding $I_{CSD}$s behaved similarly, as they represent the addition of all unitary somatic $I_{CSD}$. The explanation lies on the fact that the somatic $I_m$ is short lasting and spiky, so that even a small activation synchrony of the firing cells avoided their temporal summation in the extracellular space. In fact, when the $I_m$ peaks were time-locked throughout the population (Fig. 4B, bottom), the aggregate $I_{CSD}$ peak also increased, and the overall waveform became more akin to that of the single-cell somatic $I_m$. Also the initial fast transient of the somatic $I_m$ was revealed on the FP (arrowhead), and a subsequent hump was uncovered (small arrow). However, the maximum PS amplitude still saturated at low $g_{Na}$ densities. It can be concluded that the temporal scatter of activation dissipates the contribution of the short-lasting somatic $I_{CSD}$ so that the PS peak occurs at an instant when the FP at the st. pyr. is additionally contributed by slower current components (see following text). Most relevant is that a much larger $g_{Na}$ was required to fairly reproduce the antidromic AP of real cells than to achieve the maximum antidromic PS. At best, this reached about half the experimental value (10–12 vs. 24 mV), thus $g_{Na}$ cannot be considered a limiting parameter for the PS and other factors must be involved.

Dendritic action currents contribute to the PS peak at the stratum pyramidale

Somata and dendrites of pyramidal cells are segregated spatially, allowing the study of their relative contribution to the PS shape. We used a small aggregate (256 units) to minimize the contribution of the dendrites at the st. pyr., and no scatter of activation to avoid reduced temporal summation of individual somatic currents (Fig. 5A, left). In this case, the late hump became residual (compare to Fig. 4B, bottom left), and the overall PS waveform followed a time course similar to the individual somatic $I_m$ (compare with Fig. 4A, right). The almost complete dominance of somatic currents also was revealed by the fact that the PS peak did increase with $g_{Na}$, matching the single-cell somatic $I_m$ behavior and the ensemble $I_{CSD}$s in absence of temporal scatter (curved arrows in Figs. 4 and 5). These computations indicate that the uncovered delayed hump using large aggregates (Fig. 4B) is caused by volume propagated currents from neuron elements other than somata, i.e., dendritic currents.

If the spatially clustered somata were the only active generators, the size of the aggregate should affect the FP amplitude but not its waveform because all somata generate identical current waveforms and the extracellular space has no capacitive effects. However, this was not the case, and the larger the cell aggregate, the more conspicuous was the late hump in the st. pyr. (Fig. 5B) and the longer was the overall half-width of the PS. This is the expected result when two spatially segregated generators yielding different current waveforms are activated simultaneously. The FP waveform recorded among somata changed with the size of the aggregate because of increased volume-propagated dendritic action currents reach-
ing the st. pyr. The dendritic hump, undetected for the smallest aggregate (asterisk), gradually grew even larger than the somatic initial fast transient, so that the PS peak occurred significantly later. Naturally, with temporal scatter of the activated cells the peak time also will occur at an instant strongly contributed by dendritic currents. Hence measurement of real PSs at the st. pyr. is strongly contaminated by dendrites.

**Contribution of volume-propagated currents to the FP in different strata**

Along the region where the AP is actively conducted, from the st. or. to the st. rad., the duration of the ensemble current sink was always shorter than the corresponding negativity in the FPs (Fig. 6, left, thick vs. thin tracings). In the st. pyr., the peak of the $I_{\text{CSD}}$ always led that of the PS (~0.15 ms), whereas the opposite occurred 150 µm below in the st. rad (Fig. 6, left, vertical dashed lines). A gradual transition was observed at intermediate locations. The computations matched well the experimental results only when model neurons had excitable dendrites (compare middle and right). Phases of the FPs the negativity of which mismatched the time course of the ensemble current sink at the corresponding locations must be contributed by currents from far generators (dendrites or somata for recordings in st. pyr. and st. rad., respectively). In a model of passive dendrites, there is no dendritic contribution to the st. pyr., so that FP and CSD match in duration (top right). Also volume-spread only occurs from the active soma in the st. pyr. to the passive dendrites in the st. rad., which shows the corresponding negativity (arrow in bottom right) in clear contrast to the absence of local sink. These results illustrate that the peak and late phase of the PS at the st. pyr. are contributed and dominated, respectively, by dendritic volume-propagated currents.

_Fig. 6._ Contribution of volume propagated currents to the FP in different strata. In experimental recordings (left), the superposition of the FP (thick tracings) and $I_{\text{CSD}}$ (thin tracings) showed different time courses at any extracellular location. Negativity in the FPs was always longer than the corresponding sink (see asterisks). In the st. pyr., the local currents (contributed by somata) peaked earlier than the PS (note vertical dashed lines). The opposite occurred at the st. rad. Same result was obtained in the model only when neurons were endowed with excitable dendrites (compare middle and right). Phases of the FPs the negativity of which mismatched the time course of the ensemble current sink at the corresponding locations must be contributed by currents from far generators (dendrites or somata for recordings in st. pyr. and st. rad., respectively). In a model of passive dendrites, there is no dendritic contribution to the st. pyr., so that FP and CSD match in duration (top right). Also volume-spread only occurs from the active soma in the st. pyr. to the passive dendrites in the st. rad., which shows the corresponding negativity (arrow in bottom right) in clear contrast to the absence of local sink. These results illustrate that the peak and late phase of the PS at the st. pyr. are contributed and dominated, respectively, by dendritic volume-propagated currents.

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Effect of channel distribution along the somatodendritic axis: optimizing the neuron prototype

The results thus indicate that active currents from both soma and dendrites contribute to shape the PS at any recording position. Further optimization of the neuron prototype required that the spatial waveform of APs and PSs along the somatodendritic axis was considered as a whole. We have tested a variety of Na\textsuperscript{+} channel densities and distributions, summarized in Fig. 7. In a neuron with passive dendrites (type 1, Fig. 7, 1), the AP presented a fast decay along the apical shaft and negligible $I_m$ in this region. When used in the aggregate model, this prototype unit yielded an unrealistic antidromic PS as noted by the very fast decline in the proximal apical shaft and the absence of extracellular source-sink sequences (see $I_{CSD}$). The small $I_{CSD}$s (arrowhead) are due to capacitive currents during the passive spread of the AP (note the miniature compartmental currents on the $I_m$ plots). A relatively high $g_{Na}$ was needed at the soma and IS/AH to obtain an AP rising slope within the experimental values and without AB brake.

When using excitatory dendrites, an important feature to reproduce is the well-known decremental conduction of APs with increasing duration along the apical shaft (Leung 1978; Turner et al. 1991). We first checked homogeneous $g_{Na}$ density throughout soma and dendrites (Magee and Johnston 1995a,b; Spruston et al. 1995) and proportional $g_{Na}$. The neuron type 2, shown in Fig. 7, 2, had $200 \text{ pS/\mu m}^2$, which is two to three times higher than suggested by experimental findings. In this case, a hot spot in the IS ($5 \text{ \mu m}$ long, $50 \text{ \mu m}$ away from the soma in the example of the figure) was required for somatic AP invasion. This type of neuron yielded unrealistic results in several ways. First, the somatic AP always presented a prominent AB-brake (arrowhead in Fig. 7, 2), indicating a delay on the IS-to-soma invasion that was never apparent in actual recordings (Fig. 1). The AB-brake can be concealed acceptably only when the hot spot is placed near the soma. Second, the AP rate of rise was very slow ($<200 \text{ V/s}$) and the half-width too large ($>1 \text{ ms}$), both far from the measured range of the in vivo APs. Lower $g_{Na}$ densities ($<80 \text{ pS/\mu m}^2$) (e.g., Stuart and Sakmann 1994) yielded even slower APs or failed to invade altogether. Third, decremental AP conduction was not achieved, and backpropagation was too slow (see $V_m$ plots, Fig. 7, 2).

FIG. 7. Somatodendritic distribution of Na\textsuperscript{+} channels determines the shape of the AP and the PS profile. Four examples of spatial distribution of Na\textsuperscript{+} channels along the axon, soma, and apical dendrites are shown. $g_{Na}$ per surface unit is indicated in the top schemes (pS/\mu m\textsuperscript{2}). A passive-dendrite neuron (type 1) with high $g_{Na}$ at the soma and initial segment/axon hillock (IS/AH) fires an antidromic AP that spread passively into dendrites ($V_m$). Addition of capacitive currents from multiple dendritic compartments can be observed in the cumulative $I_{CSD}$ (arrowhead). In a neuron with homogeneous low $g_{Na}$ and a hot spot in the IS (type 2), delayed soma invasion is noted by an AB-break in both AP and PS (arrowheads), and nondecremental AP backpropagation occurred. Decremental conduction along the somatodendritic axis can be achieved using a somatopetally decreasing gradient of $g_{Na}$ (type 3) or an increasing gradient of $K_g$ channels (type 4), although in the latter the AP half-width barely increased. AP speed of invasion was too slow in all cases, and some features of the antidromic PS spatiotemporal map clearly differ from the experimental. See RESULTS for further details and comparisons.
Fourth, the model FP yielded unrealistic PSs, presenting a pronounced inflection on the negative-going phase, caused by the slow invasion of IS-currents (arrowhead in Fig. 7, FP), and half-widths about twofold the experimental values. Attempts to correct the deviations were made by increasing the somatic $g_{Na}$ (900 pS/m$^2$) and using a somatofugally decreasing $g_{Na}$ gradient (Fig. 7, FP). This and many other combinations of distribution and kinetics of this channel yielded an acceptable attenuation of the AP along the apical dendrites (arrow on $V_m$ plots) in parallel to the increase of its half-width, but the speed of invasion was still too slow ($0.3$ vs. $0.47$ m/s in vivo, measured on $I_{CSD}$s). Also, the half-width of the PS was too large ($1$ vs. $0.7$ ms in vivo), and the decay rate of the PS negativity along the apical dendrites was too slow, showing delayed positive-going phases (circle-headed arrow in FP plots). Some of these divergences could be ameliorated by using a somatofugally increasing gradient of the A-type K$^+$ channels (Hoffman et al. 1997) and homogeneous $g_{Na}$. The neuron type 4 shown in Fig. 7, FP yielded a PS with a faster falling rate of the negativity along the apical tree (arrow in FP plots) and shorter source-sink sequences. However, the AP speed of invasion along the apical shaft decreased ($0.2$ m/s) and its half-width increased only slightly. Increasing $g_{Na}$ accelerated the speed of the AP, but it always felt short of the in vivo values and failed to improve noticeably other features of the antidromic PS.

In general, the higher the $g_{Na}$ density in dendrites the larger was the PS in the st. rad., in contrast to the rapid saturation found for the PS at the st. pyr. (compare the FP plots for neuron types 1–3). This was caused by the longer duration and smoother waveform of the $I_m$ in dendritic compartments than somata, for it is barely affected by the scatter of the activation. The precise waveform of the PS at this region can be strongly modified by the density and distribution of K$^+$ channels (compare the FP plots for neuron types 3 and 4).

Some single-cell electrotonic parameters had a notable impact on both AP and PS waveforms. Varying membrane resistivity from 17 to 140 k$\Omega \cdot$ cm had negligible effects. As expected, varying membrane capacitance from 1.5 to 0.375 $\mu$F/cm$^2$ caused an increasing velocity of APs and PSs.
progressively invading longer distances. Still, the PS at the st. pyr. increased <10%.

By contrast, internal resistance \((R_i)\) had a strong impact on the antidromic PS at any location. All preceding simulations employed a \(R_i\) of 150 \(\Omega \cdot \text{cm}\), which is usual in the literature. Figure 8 shows the comparative results for \(R_i\) of 200, 100, and 50 \(\Omega \cdot \text{cm}\) (thick, medium, and thin tracings, respectively). Lowering \(R_i\) caused faster AP propagation and a slight amplitude decrease (Fig. 8A). However, the corresponding model antidromic PS increased almost linearly to \(1/R_i\) (~400%, Fig. 8, B and C). A notable decrease of the PS half-width also was observed (1.1 vs. 0.8 ms in the st. pyr. for 200 and 50 \(\Omega \cdot \text{cm}\), respectively), getting closer to the in vivo PS. The plots of the PS amplitude shown in Fig. 8C also show a much faster decay of the negativity along the apical dendrites. Note the progressive flattening of the plots and the somatofugal shift of the maximum negativity with higher \(R_i\). This result is explained by the different AP speed along the somatodendritic axis. During fast APs, inward currents along invaded dendrites are better time-locked, enabling their addition in the interstitium and increasing the FP. Because this is contributed by far generators from different strata, this increment of negativity clustered around a specific stratum, with the maximum shifting toward the location of stronger generators (see arrows in Fig. 8C). On the other side, slow APs have inward currents progressing in a sequential manner, reducing their temporal addition in the extracellular space.

These results at the ensemble level can be accounted at the single-cell level. The slight variations observed in \(V_m\) at the soma (Fig. 8A) are associated to moderate changes of capacitive currents \((I_{cm})\) and to notable increments of ionic currents \((I_{m})\) and hence of \(I_m\), with the lower \(R_i\) (Fig. 8D). The increment of \(I_m\) also is reflected in the corresponding aggregate \(I_{CSD}\) (Fig. 8E). These computations indicate that a low \(R_i\) fit most of the discrepancies of the model and experimental antidromic PS spatiotemporal map that we could not achieve by altering other significant parameters.

**DISCUSSION**

This study discloses the relevant parameters contributing to the amplitude and shape of the PS. As for any other FP, the PS is built up by the addition of near (local) and far generated (volume propagated) membrane currents. The selective clustering of somata and dendritic elements in different strata, and the different waveform and timing of their respective \(I_m\) during APs, constitute the basic factors shaping the PS. The role of any other parameter will depend on the capacity to define or modulate the timing and relative contribution of somatic and dendritic currents to the extracellular space.

**Can the aggregate model restrict the variability of experimental measurements?**

Except for specific details attributable to species differences or intact versus in vitro preparations, the overall features of the antidromic PS spatiotemporal map coincide with previous reports (Leung 1979; Miyakawa and Kato 1986; Sperti et al. 1966). The extraordinary steadiness of this evoked potential facilitated the parametric restriction at the single-cell and subcellular level, the experimental data of which are more disparate.

The main handicap for model studies is that the experimental measurement of single-cell and subcellular parameters shows large standard errors. The transcendence of this variability gains practical significance when the experimental values are extrapolated at a large scale so as to predict their role on the global electrical behavior of neurons or cell ensembles, as we made in this study. In the aggregate model, the AP parameters have been used to restrict the useful ranges of channel density, kinetics and distribution, to obtain the spatial map of \(I_{m}\), in a way similar to voltage clamping of a cell with AP waveform commands along its entire morphology. Once obtained, the spatial map of \(I_{m}\), the precise set of subcellular parameters, becomes irrelevant for the subsequent calculation of FPs as long as these fitted the experimental PS. In consequence, the combined reconstruction of real PSs and APs is a powerful tool to restrict the useful ranges of subcellular parameters that should expectedly match those experimentally obtained. However, a quick survey of the available literature is enough to realize that in many cases data obtained from different labs are only roughly similar but, given their transcendence for cell function (and modeling), far from reliable. Whether this is due to low technical resolution or actual variability is not clear. If we consider the wide experimental ranges as reflecting functional cell heterogeneity, we are bound to admit striking differences within a homogenous population of neurons. For instance, the range of AP rising rates found in our neuron sample would require up to sixfold the somatic \(g_{Na}\) calculated in a pyramidal model cell (Fig. 4), a range too wide to be physiological. On the contrary, we might consider that data dispersion is caused by a variable interference of recording devices with ongoing physiology due to mechanical stress (e.g., Somjen et al. 1993). In this case we should consider the faster APs the more realistic and representative of the cell population. Unfortunately, the low dispersion found for the PS amplitude cannot be used to decide this issue as it is an averaged population response. As long as this matter is not clarified, we arbitrarily considered the higher values as the more representative of an average pyramidal cell. In consequence, we developed our aggregate model by assuming an admittedly questionable homogeneous electrical behavior for each cell in the population.

Somewhat surprising was the finding that the ISs do not appear to contribute a significant amount of current. Field compound negative spikes have been ascribed to IS currents in the CA1 (Sperti et al. 1966) (see Fig. 1, asterisk) and other structures (Lorente de Nó 1947b). Earlier CSD studies also failed to discriminate a current sink distinct from that attributed to cell soma (Herreras 1990; Leung 1979; Miyakawa and Kato 1986), indicating that the mentioned spike is actually generated in the st. pyr. border and spreads toward the st. oriens by volume conduction. On the contrary, we are reporting for the first time a current sink that may be attributed to active axon generators in the alvear region. The present experimental study shows no leading passive currents for the initial sink at the st. pyr. nor does the somatic APs display an AB break, indicating a high safety factor for AP invasion of the soma and negligible IS-to-soma delay. When we used neuron prototypes with high \(g_{Na}\) density at the IS, the resultant antidromic AP always showed a prominent AB brake, and this also was

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**SHAPING OF CA1 POPULATION SPIKES**

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appreciated on the model antidromic PSs. Because this is contrary to experimental findings, it has to be inferred that the IS $g_{Na}$ density needs to be not much higher than at the soma. Interestingly, a recent report found low density of Na$^+$ channels in ISs of subicular pyramidal cells, comparable with that of soma and dendrite membranes (Colbert and Johnston 1996).

Among the more relevant experimental values for this study are those concerning dendrites. Available data are barely quantitative, and only gross qualitative features can be reproduced. Although a decremental AP invasion indicates a decreasing number of activated Na$^+$ channels, the obtained values can be influenced markedly by geometric and electrotonic factors and does not necessarily mean a decreasing Na$^+$ channel density. Certainly, decremental APs run with a decreasing $I_{m}$, but this may also result from an homogeneous but low $g_{Na}$ density unable to support fully regenerative APs (see Johnston et al. 1996). Also the cumulative effect of branching, an increasing density or faster kinetics of repolarizing channels (Hoffman et al. 1997) or synaptic impingement (Tsubokawa and Ross 1996) may cause decremental conduction.

Whether Na$^+$ channel density is homogeneous or not throughout the somatodendritic membrane cannot be decided from the current model results, but in our opinion, it cannot be rigorously concluded from the available data obtained from studies on membrane patches either. The number of estimated channels varied enormously along the apical shaft (e.g., Magee and Johnston 1995a; Stuart and Sakmann 1994), what may be interpreted similarly as sampling dispersion or as the result of variable mechanical disruption of functional channels. Somatofugally decreasing $g_{K}$ and increasing $g_{Na}$ are not mutually exclusive, and some functional aspects, such as AP attenuation, can be achieved in both ways. Whereas the latter appears well established (Hoffman et al. 1997) and the $g_{Na}$/$g_{K}$ ratio may be the most important parameter shaping dendritic APs, a moderately decreasing gradient of $g_{Na}$ may be necessary for a correct increase of its half-width. Further tuning along the apical shaft requires precise experimental measurement of AP parameters. Our model can be used then in an iterative manner to find suitable $g_{Na}$ distributions by tuning the single-cell channel densities and distributions and comparing the model to the experimental PS profile.

In any case, it became obvious that low $g_{Na}$ densities (<200 pS/μm²) enable dendritic AP invasion but much higher densities (500 pS/μm²) are needed to generate realistic APs at the soma as recorded in vivo. The speed of APs was too slow even with the higher $g_{Na}$. Curiously, most single-cell models for cortical or hippocampal pyramidal neurons use $g_{Na}$ 4–10 times higher than that calculated in membrane patches to obtain acceptable somatic APs. It is worth mentioning that the parameters of APs measured in vitro (e.g., Storm 1987) are much slower than in vivo, and closer to those reproduced by the neuron type 2, so we can speculate that Na$^+$ currents are somehow depressed in nonintact preparations, as it happens in immature cells (Cummins et al. 1994; Spigelman et al. 1992) or low-temperature recordings (Thomson et al. 1985). A putative decrease of Na$^+$ channel availability or slower kinetics should entail remarkable changes in local excitability. This possibility is supported by the fact that APs are much more easily initiated in the apical shaft of CA1 pyramids in vivo (Andersen and Lømo 1966; Fujita and Sakata 1962; Herreras 1990) than in vitro (Turner et al. 1991; see Johnston et al. 1996 for a review), suggesting changes in the local AP threshold, also defined by Na$^+$ channel availability. No doubt this matter deserves further attention because computational properties are strongly dependent on the fine modulation of local dendritic excitability.

The results obtained with varying $R_{i}$ are the clearest example of the great sensitivity of the aggregate model to unveil the role of some subcellular parameters the effect of which may go unnoticed at the single-cell level. On one side, it is evidenced the advantage of fitting the experimental PS by an aggregate model over the low resolution of single-cell models fitting APs. On the other, two important practical implications can be derived. First, minor changes of $V_{m}$ measured in single cells actually can be associated to large $I_{m}$ variations (note the negligible difference of the antidromic APs between soma and ap1 compartments in Fig. 7), leaving unnoticed important functional changes during experimental manipulations and causing misinterpretation of the data. Second, an effort to improve the accuracy of the experimental measurement of electrotocic parameters is required: the constancy of experimental PS amplitude is not compatible with the dispersion of available single-cell measurements.

Volume propagation: from evoked field potentials to elementary currents

The factors shaping PSs may be grouped in two classes, those we called subcellular, which define/modulate the spread and magnitude of axial and transmembrane currents along the entire morphology of individual cells, and those defined as macroscopic, which concern the spread of currents in the extracellular space. Among the latter, tissue resistivity and spatial and temporal dispersion of activated membrane generators are the most relevant. For the shake of simplicity, we have neglected the possible effects of anisotropy, which may cause important modulations in the spread of extracellular current (Holsheimer 1987; Okada et al. 1994). It is usually thought that APs contribute little to evoked FPUs due to insufficient synchrony during activation and/or the canceling of action currents in the interstitial space due to irregular spatial arrangement of the activated neuron elements (see Mitzdorf 1985 for a review). Although the strong stratification of the CA1 region offers the most favorable conditions and indeed allows distinct PSs to develop, neither synchrony nor spatial arrangement are ideal. We found that the experimental variability of these basic parameters did not cause a notable impact on the model PS amplitude (Fig. 3). Although this may appear paradoxical, it is in fact due to the equalizing influence of volume-propagated currents from different membrane generators. The scheme in Fig. 9 outlines some relations between different levels of complexity to illustrate the relative contribution of somata and dendrites to the PS. Even if the temporal scatter is not a major modulator in itself, it has a critical role in limiting the role played by other factors. The short duration and spiky waveform of somatic action currents makes the temporal scatter to cause a strong decreasing effect on the ensemble somatic sink (see also Fig. 3, C and D) by reducing the temporal coincidence required to sum up in the extracellular space. However, the PS was barely sensitive to temporal scatter at the time of the peak (Fig. 3C), indicating that other currents from far generators have a substantial contribution at that time. These are necessarily of dendritic origin, the extracellular...
summation of which is barely modified by the small latency variability due to their longer and smoother waveform. The currents generated by far somata will increase the amplitude but shall not change the waveform. The fast somatic currents thus define the slope of the initial negative-going limb, whereas dendritic currents have an increasing dominance during the time span of the PS that is already strong at the PS peak and may even account for the whole negativity during the rising limb (Figs. 4 and 5) (see also López-Aguado et al. 2000). Moreover, because of the obligatory delay of dendritic currents during backpropagation, the increasing contribution is defined also spatially so that proximal dendrites contribute more at earlier times than distal dendrites. In turn, the waveform of individual somatic \( I_{\text{m}} \) is sharp in correspondence to the large amplitude and rate of rise of somatic APs (Fig. 9). These are two key AP parameters governed by inward Na\(^+\) currents, limited by the driving force, channel kinetics, and active repolarizing currents.

Among the macroscopic factors fostering the PS amplitude are the number of firing cells, the aggregate size, and the \( R_s \). The former two may change also the waveform of the PS, especially at the st. pyr., by altering the relative dominance of somatic and dendritic generators (Figs. 3–5). The bigger the aggregate, the larger the relative contribution of dendrites, which may be relevant to the slice preparation that contains a reduced number of cells. This effect was revealed as the appearance of a delayed hump in the somatic PS in absence of temporal scatter of activation and originated by the different time course of somatic and dendritic currents. If these were similar but slightly delayed, the changes in the somatic PS waveform would be minimal because far somata and far dendrites would contribute with currents of identical time course. It can be inferred easily that the poor temporal summation of somatic currents makes volume-propagated currents from dendrites to gain more relevance at the time of the peak. Thus latency variability among firing cells indirectly favors dendritic contribution the bigger the cell aggregate is because far somata also will contribute less than far dendrites in spite of similar distance to the recording point. It should be mentioned that the peak of the PS in absence of temporal scatter occurs much earlier, whatever the size of the aggregate, as it is almost entirely dominated by the phase-locked spiky somatic currents that peaked at an instant when the AP is just initiating in dendrites. It is the dissipating effect of latency variability on the ensemble somatic currents that makes the peak to be delayed at later times when dendritic contribution is larger. In this sense, even if the natural effect of the latency variability is reducing the ensemble somatic currents, the decrease of the corresponding PS at the st. pyr. occurs at a smaller rate because of the increased weight of dendritic contribution.

Somewhat surprising is the finding that the Na\(^+\) channel density is a critical factor for the antidromic PS amplitude at the st. rad. but not at the st. pyr. The explanation lies on the larger amplitude and rising slope of the somatic than dendritic APs (Andreasen and Lambert 1995; Turner et al. 1991), making the \( I_{\text{m}} \) of the former short lasting and limited by the Na\(^+\) driving force. It can be argued that the dendritic \( g_{\text{Na}} \) in some computations was smaller than in somata. However, the used densities are only an optimized parameter adjusted to reproduce the limiting factors, namely the experimental somatic APs and the known decrease of backpropagating APs (Turner et al. 1991). Further, when the \( g_{\text{Na}} \) was set homogeneous through the entire somatodendritic axis and the features of dendritic APs shaped by somatopetally increasing the K\(^+\) channel density (Fig. 6–8) (see Hoffman et al. 1997), the behavior of the dendritic \( I_{\text{m}} \) s was the same, an unexpected result that confirms the limiting effect of the AP amplitude and duration for local inward currents, whichever the channel assortment.

Different distributions of somatodendritic channel densities strongly define the antidromic PS at the st. Neither den-
dritic AP parameters cause the saturation of inward currents (as it happens in somata) nor their smooth and longer waveform allows a sensitive reduction of the ensemble extracellular currents by temporal scatter. Yet, the PS amplitude at the st. pyr. changes <20% for extreme cases of dendritic channel densities (Fig. 6), even when dendritic contribution to this layer has been estimated much larger (~30–40%; see RESULTS) (see also López-Aguado et al. 2000). Comparing $I_{\text{CSD}}$ in the st. pyr. of aggregates with active and passive dendrite neurons, we found that the decreased contribution of local somatic currents in the former was compensated by volume-propagated dendritic currents. This can be understood in terms of the reciprocal shunt between two near membrane generators simultaneously activated. The larger and longer $I_{m}$ contribution to the extracellular space of somata in passive dendrite neurons is compensated by the addition of dendritic currents volume-propagated up to the st. pyr. in the model with excitable dendrites. The strong differences in duration and shape of somatic $I_{m}$ in the two neuron types are clear evidence of the interaction between somata and dendrites. The reciprocal shunt between adjacent active membranes cause a powerful reshaping of the magnitude and time course of their respective $I_{m}$s.

Quantitatively, much more important than the magnitude of subcellular $I_{m}$s is their temporal relationship. The wider the temporal overlap of somatodendritic currents the larger will be the instantaneous current at the extracellular space and hence FPs. Temporal overlap of subcellular currents is greater the faster is the AP backpropagation. Increasing dendritic channel density is far less effective than lowering $R_{i}$ (Fig. 8). This parameter controls the electrotransit spread of currents so that the lower $R_{i}$ is, the farther and faster axial currents can spread, activating newer dendritic regions that will add more currents to the extracellular space. Most of the experimental and model PS discrepancies were eliminated by using a low $R_{i}$, which all were related to the speed of AP backpropagation. In the experimental CSD analysis, we measured a much faster speed than could be achieved by using the values for somatodendritic $\tilde{g}_{\text{Na}}$ densities, channel kinetics, and electrotransit constants found in the literature. The optimum $R_{i}$ value here used in the late part of this study is in agreement with that recently reported by Stuart and Spruston (1998) of $\sim$75 $\Omega \cdot$ cm.

Functional implications of the contribution of dendritic currents to the PS

Because dendritic action currents during AP invasion are barely susceptible to the latency variability of activation among firing cells, their contribution to the st. pyr. by volume propagation confers stability to the PS amplitude on changes of the latency dispersion. This result argues against the extended idea that desynchronization can be a powerful decreasing factor of the hippocampal PSs. Indeed, it seems not to be the case, at least for antidromic activation. In fact, sizable reduction only could be obtained when the average dispersion in the population was 1 ms (beyond 2 ms for the latest neurons), which is too large for antidromic activation of pyramidal cells endowed with fast conducting axons (Lipsky 1981). It should be remembered that the half-width of the PS is only 0.6–0.7 ms, and the duration of the ensemble somatic sink even shorter (~0.4 ms). On the other side, the PS at the st. pyr. becomes susceptible to the proper lability of dendritic action currents. It is known that AP dendritic invasion can be modulated in a variety of experimental paradigms (Callaway and Ross 1995; Herreras and Somjen 1993; López-Aguado et al. 2000; Mackenzie and Murphy 1998; Spruston et al. 1995). In these situations, the soma and dendrites of individual cells change the timing, magnitude, and spatial distribution of their current contribution to the extracellular space, making unreliable the interpretation of the PS (see López-Aguado et al. 2000 and following text).

In a strict sense, the unavoidable mixed contribution of currents from somata and dendrites makes the PS to depend on the precise timing of the currents between two interdependent membrane generators. Because the AP actively propagates along the somatodendritic axis (Herreras 1990; Leung 1979; Turner et al. 1991), the magnitude of the $I_{m}$ at a specific membrane locus will depend on the speed and direction of the AP and on the electrotransit status so that variations of the AP speed, the locus of AP initiation, and the concurrence of synaptic activity will cause strong modulations of the magnitude and timing of $I_{m}$ at different compartments (Varona et al. 1998).

In practice, the fact that dendritic currents contribute to the PS at the st. pyr. may not alter the widespread notion that its amplitude is proportional to the number of activated cells. However, this only holds true for a control PS. Once it is altered by any experimental manipulation or ongoing activity, it cannot be ruled out that the changes are due to the unbalance of the relative contribution of somatic and dendritic currents or their timing, regardless of the number of firing cells (López-Aguado et al. 2000). Double recordings at the st. pyr. and st. rad. may help to explain PS variations at the st. pyr. because most modulations will differently modify the PS in both strata.

Note on aggregate versus single-cell models

Our results modeling the FP and CSD profiles along the entire neuron morphology show that this is a unique method to integrate modeling and experimental techniques to draw reliable conclusions when interpreting physiological data. Several advantages over single neuron models are as follows: 1) the experimental paradigm (PS) is obtained from intact unclamped cells, allowing normal interplay of ion conductances along the entire cell morphology; 2) its aggregational nature makes it extremely sensitive to minor changes of some individual cell parameters obtained from partial single-cell studies, limiting the useful range of, for instance, channel properties and distributions obtained in nonintact preparations; 3) it enables robust predictions of channel density at each loci; and 4) because the paradigm is a well-established macroscopic experimental data, it can be used as a benchmark to any other single neuron model and eventually even to test the accuracy of measured subcellular experimental variables.

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