# Generation of Sustained Field Potentials by Gradients of Polarization Within Single Neurons: A Macroscopic Model of Spreading Depression

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Makarova J, Makarova VA, Herreras O. Generation of sustained field potentials by gradients of polarization within single neurons: a macroscopic model of spreading depression. J Neurophysiol 103: 2446-2457, 2010. First published March 10, 2010; doi:10.1152/jn.01045.2009. Spreading depression (SD) is a pathological wave of depolarization of the neural tissue producing a negative macroscopic field potential  $(V_{o})$ , used as a marker for diagnostic purposes. The cellular basis of SD and neuronal mechanisms of generation of  $V_0$  at the microscopic level are poorly understood. Using a CA1 mathematical model and experimental verification, we examined how transmembrane currents in single cells scale up in the extracellular space shaping  $V_{0}$ . The model includes an array of 17,000 realistically modeled neurons (responsible for generating transmembrane currents) dynamically coupled to a virtual aggregate/extracellular space (responsible for  $V_{o}$ ). The SD wave in different tissue bands is simulated by imposing membrane shunts in the corresponding dendritic elements as suggested by experimentally assessed drop in membrane resistance. We show that strong isopotential depolarization of wide domains (as in the main SD phase) produce broad central cancellation of axial and transmembrane currents in single cells. When depolarization is restricted to narrow dendritic domains (as in the late SD phase), the internal cancellation shrinks and the transmembrane current increases. This explains why in the laminated CA1 the  $V_{o}$  is smaller in the main phase of SD, when both dendritic layers are seized, than in the SD tail restricted to an apical band. Moreover, scattering of the neuronal somatas (as in cortical regions) further decreases the aggregate  $V_0$  due to the volume averaging. Although mechanistically the  $V_0$  associated to SD is similar to customary transient fields, its changes maybe related to spatial factors in single cells rather than cell number or depolarization strength.

### INTRODUCTION

Sustained field potentials have long been documented in the brain although their biophysical origin is to a large extent still a matter of speculation. One of the best known macroscopic phenomena is the slow potential change associated with spreading depression (SD) (Leão 1944, 1951). SD is a slowly propagating wave of electric activity in nervous tissue that has an important clinical impact, not least because it is related to the auras of migraines (Fabricius et al. 2006) and the extension of neuron death in the ischemic penumbra (Iijima et al. 1992; Largo et al. 1996). Most studies of this phenomenon focus on its remarkable macroscopic features, including the strong changes in ion concentrations across large areas of tissue and the associated negative giant extracellular potential ( $V_o$ ). However, the microscopic mechanisms underlying these macro-

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scopic changes are not fully understood (for review, see Bureš et al. 1974; Somjen 2001). Here we have developed a biophysical model to explore the generation of the aggregate potential on a subcellular basis using the core conductor and field theories, and we have tested its main predictions experimentally.

Early recordings in neuronal somata during SD described a vanishing transmembrane potential  $(V_m)$  and resistance  $(R_m)$ , and the loss of electrical responsiveness (Sugaya et al. 1975). These single-cell data fit reasonably well within the mechanistic description of SD as a macroscopic extracellular wave of excitatory agents (Grafstein 1956). However, such conceptual framework does not provide a formal explanation linking all the time evolving variables, such as, e.g., neuron depolarization or the flood of potassium, to the associated negative  $V_{0}$ . Several theories have been proposed to explain the SD-related negative  $V_{0}$ , involving different anatomical substrates and mechanisms, such as differential polarization of neuronal domains (Canals et al. 2005; Leão 1951; Wadman et al. 1992), glial-mediated syncytial currents (Sugaya et al. 1975; Tomita 1984), and Donnan-like electrodiffusion potentials (Almeida et al. 2004). There is also increasing experimental evidence that neurons shape the  $V_{\rm o}$  (for review, see Herreras et al. 2008; Somjen 2001), although a convincing unitary to macroscopic description has not yet been formulated.

The major handicap has been to find an appropriate generator for extracellular currents inasmuch as neurons were thought to be electrogenically inactivated. Indeed isopotential cell conductors, whether depolarized or at rest, cannot generate net transmembrane currents (Im's) and hence, they cannot produce and sustain the observed negative  $V_{0}$ . This apparent puzzle was recently resolved by analyzing intradendritic recordings, showing that cell depolarization is neither complete nor homogeneous in CA1 pyramidal neurons during SD (Canals et al. 2005). While the somata always depolarize, longitudinal gradients of intracellular depolarization, and membrane shunting extend along different somato-dendritic regions, and the distal dendritic zones remain electrogenically active. These single-cell features correlate tightly with the stereotypic spatial evolution of the negative  $V_0$  that differs among strata (Herreras and Somjen 1993a). Current-source density (CSD) studies of such potential profiles (Makarova et al. 2008) show during the main phase of SD a striking distribution of inward currents in the outer domains of dendritic trees flanking wide zones of zero current. These results suggest that subcellular interactions within the main somato-dendritic body may be essential for  $V_{0}$ dynamics. However, defining the relationship between singlecell electrogenesis and macroscopic fields is not trivial given

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the influence of the complex core geometry on the intracellular distribution of currents and the volume conduction of currents in the extracellular space (López-Aguado et al. 2002; Rall and Shepherd 1968; Varona et al. 2000). To establish rigorous causal relationships, a comprehensive platform is required to simultaneously monitor subcellular and macroscopic electrical variables in time and space. At the moment, this can only be effectively achieved by biophysical simulations.

Different models of SD have focused on its initiation, propagation, and ion redistribution (Dahlem and Hadjikhani 2009; Kager et al. 2000, 2002; Shapiro 2001; Tuckwell and Miura 1978). However, little attention has been paid to the reproduction of the associated  $V_{o}$  and to its subcellular basis. Notably, the latter requires anatomically realistic definitions of the units and population as well as a combined formal handling of unitary and field electrogenesis to deal with the intra- and extracellular currents. Earlier we studied the contribution of different membrane channels to the electrical state of membranes in single cells during SD (Makarova et al. 2007). Here we analyze how this relates to the macroscopic  $V_0$  in three steps. First we explore the spatial distribution of currents and potentials within a single cell when it is subjected to SD-like depolarizing conditions across different extensions of its anatomy. Second, we scale up the estimated  $I_{\rm m}$ 's to a threedimensional (3-D) macroscopic model of the CA1 to estimate the aggregate  $V_{\rm o}$ . Finally, we test experimentally the main predictions of the subcellular to macroscopic relations found in the model. We have used a steady-state adaptation to our former virtual 3-D multineuronal model employed to simulate transient evoked field potentials (López-Aguado et al. 2002; Varona et al. 2000), further modified to include  $V_0$  feedback to correctly calculate the compartmental  $I_{\rm m}$ 's under conditions of SD. The SD-related currents in single cells were previously derived by fitting the experimental evolution of  $V_{\rm m}$  and  $R_{\rm in}$ during SD along the cell's anatomy (Makarova et al. 2007). The estimates from the model and the experimental tests indicate that the stereotypic changes in the spatial profile of the hippocampal  $V_{o}$  during SD are well explained by the differential polarization of pyramidal neurons. The dominant factors defining the spatial distribution of unitary  $I_{\rm m}$ 's and hence the characteristic shape of the aggregate  $V_{\rm o}$  are the cancellation of axial currents within single cells, the fine geometry of subcellular elements, and the orderly parallel arrangement of units in the aggregate.

### METHODS

### Model system of SD

GENERAL ARCHITECTURE. This study pursues the exploration of the subcellular basis of SD and associated  $V_o$ . At a given instant of time,  $V_o$  depends on the instantaneous current density and the resistivity of the extracellular space. The former is the sum of the  $I_m$ 's, which in turn depend on  $V_o$ , hence closing the feedback loop current-field-current. Thus the model system of SD includes reciprocally coupled single-cell model (responsible for  $I_m$ 's) and aggregate model (responsible for  $V_o$ ), and also membrane shunts to simulate SD-like conditions.

Because the relevant variables to calculate compartmental  $I_{\rm m}$ 's (ion concentrations, permeabilities, and  $V_{\rm m}$ ) change much slower than the channel kinetics (seconds vs. milliseconds time scale), we used the quasi-static approximation. The important advantage of such approach is that multiple variables slowly modified during SD can be neglected

or set constant as they are all compiled within the instantaneous spatial maps of  $V_{\rm m}$  and  $R_{\rm in}$  known from intradendritic studies of pyramidal cells (Canals et al. 2005). To attain the steady state, each simulation was run until the  $V_{\rm m}$  was stabilized in all compartments (Fig. 1A). To calculate the compartmental  $I_{\rm m}$ 's (Fig. 1B), we took into account the large Vo generated during the SD (Herreras and Somjen 1993a; Makarova et al. 2008), which strongly influences the magnitude and spatial distribution of the current flow along pyramidal cell membranes (see following text). The  $V_{\rm o}$  was calculated by summing all the compartmental  $I_{\rm m}$ 's at discrete points along a track of the virtual recording, weighted by distance and tissue resistivity (Fig. 1C). The experimental subcellular profiles of  $R_{\rm in}$  and  $V_{\rm m}$  were used to adjust the location, extension, and magnitude of the membrane shunt in different somatodendritic bands during depolarizing SD conditions. The net compartmental currents were derived from the steady-state opening of known channels plus an estimated contribution of a



FIG. 1. Modeling strategy to simulate the macroscopic slow field potential  $(V_o)$  during spreading depression (SD). A: a compartmental model unit was allowed to stabilize under conditions of SD (see METHODS) imposed on some of its compartments. Once a steady-state was achieved, 1 time-point (arrowhead) was chosen to estimate the  $V_m$  and  $I_m$  at that time instant. B: steady-state longitudinal profiles of  $V_m$  and  $I_m$  along a selected track of compartments spanning the entire axis of the unit model (thickened compartments, *left inset*). Dashed rectangle (*left inset*) shows SD activated compartments. C: a virtual 3-dimensional (3-D) model of the CA1 consisting of multiple identical units and positions of 16 virtual electrodes (*left*). The net transmembrane currents produce the extracellular potential  $V_o$  "recorded" at 16 virtual sites (*right*) spanning the longitudinal axis of the units.

complementary conductance (Makarova et al. 2007), termed here as  $g_{SD}$  (see following text).

Thus we have integrated our previous single-cell model optimized to reproduce standard (Ibarz et al. 2006; López-Aguado et al. 2002; Varona et al. 2000) or SD-related (Makarova et al. 2007) dendritic electrogenesis and a CA1 macroscopic cell model for hippocampal evoked field potentials (López-Aguado et al. 2002; Varona et al. 2000). This procedure permits the self-consistent system of equations to be formulated describing the closed cell-field-cell loop.

SINGLE-CELL MODELS. We employed two unit models, one of realistic branched morphology to explore specific features of hippocampal SD and another of unbranched simplified morphology that was used to explore architectonic aspects of the associated  $V_{\rm o}$ .

Simplified unit. The unit with the simplified morphology was built as a linear conductor made up of 70 identical cylindrical compartments (10  $\mu$ m long and 2  $\mu$ m in diameter) lumped one over another. All compartments had a homogeneous density of Na<sub>T</sub> and K<sub>DRA</sub> membrane channels (50 and 40 mS/cm<sup>2</sup>, respectively). In some cases, a two-compartment soma (10  $\mu$ m long and 20  $\mu$ m wide) was included at 150  $\mu$ m from one end. The electrotonic parameters were  $R_{\rm m} = 15$ k $\Omega$ ·cm<sup>2</sup>,  $R_{\rm i} = 100 \ \Omega$ ·cm, and  $C_{\rm m} = 2 \ \mu$ F/cm<sup>2</sup>, which differed somewhat from the branched neuron to compensate for unrealistic geometry. Resting  $V_{\rm m}$  was -65 mV. Compartmental  $V_{\rm m}$  was calculated as  $V_{\rm i} - V_{\rm o}$ , where  $V_{\rm o}$  was nonuniform along the anatomy and calculated as described in the following text.

*Branched unit.* For the branched unit, we started from a compartmental model of a CA1 pyramidal neuron with an average morphology (see Varona et al. 2000). The unit (287 compartments) has an average branching pattern, total dendritic length, dendritic tapering, and distribution of spine density as defined in morphometric studies (Bannister and Larkman 1995; Trommald et al. 1995). Detailed structure and dimensions of the unit model can be found at Varona et al. (2000) and //www.cajal.csic.es/Userfiles/100/file/neuronmorphlogy.pdf. The total effective area of the neuron is 66,800  $\mu$ m<sup>2</sup> (including the spine area).

Electrotonic parameters were the same as in previous models (Ibarz 2006; Varona et al. 2000). Briefly, the length of the compartments was always >0.01 and <0.2  $\lambda$ . The membrane capacitance ( $C_{\rm m}$ ) was established at 1  $\mu$ F/cm<sup>2</sup> for the soma and dendrites and at 0.04  $\mu$ F/cm<sup>2</sup> for myelinated axonal compartments. The internal resistivity ( $R_{\rm i}$ ) was 100  $\Omega$ ·cm for the soma and dendrites and 50  $\Omega$ ·cm for the axon. The membrane resistivity ( $R_{\rm m}$ ) was 50 k $\Omega$ ·cm<sup>2</sup> for the soma, 1 k $\Omega$ ·cm<sup>2</sup> for unmyelinated axonal compartments, 0.5 M $\Omega$ ·cm<sup>2</sup> for myelinated axonal compartments (surface ratio ~1:1).

The model includes 13 different types of standard ion channels simulating the active properties of CA1 pyramidal cells plus the mentioned complementary conductance employed to adjust locally  $R_{\rm in}$  and  $V_{\rm m}$  during SD. These included: three transient sodium conductances in the axon, soma and dendrites; two calcium currents (high and low threshold); one hyperpolarization-activated "h" current; and seven potassium currents. Extensive information on the literature sources, modifications and further tuning as well as the kinetic parameters and channel densities are given in the supplementary material.<sup>1</sup>

In a former study (Makarova et al. 2007), we explored in detail the contribution of depolarizing channels like the persistent Na<sup>+</sup> current and the *N*-methyl-D-aspartate (NMDA)-type conductance due to their putative sustained contribution to steady depolarization. Their contribution to the  $R_{in}$  drop during the main phase of SD was negligible, while it was more conspicuous in the late phase. Thus an additional complementary conductance of unknown nature was required to fit  $R_{in}$  values. In this study, we did not explore in detail the particular

<sup>1</sup> The online version of this article contains supplemental data.

contribution of each conductance, but the magnitude and spatial distribution of the total compartmental current responsible for  $V_{\rm o}$  production. Because  $R_{\rm in}$  compiles the activation state of all channel types, it is the only relevant parameter to compute compartmental  $I_{\rm m}$ 's at steady state. Hence we chose here not to implement those two currents and instead we used a unique complementary conductance  $g_{\rm SD}$  that encloses Na<sup>+</sup> persistent, NMDA-type and all unknown conductances. It was modeled as a user-activated, V-independent, noninactivating, and nonspecific Na<sup>+</sup>/K<sup>+</sup> conductance, as suggested by the V-clamp experiments by Czéh et al. (1993; see also Somjen et al. 2009).

The ion equilibrium potentials at rest were  $E_{\text{Na}} = 53 \text{ mV}$  and  $E_{\text{K}} = -97 \text{ mV}$ , yielding a  $V_{\text{m}} = -67 \text{ mV}$ . These result from the ion concentrations in the intra- and extracellular compartments (Somjen 2001) (see Table 1). The SD-active compartments were set with different transmembrane ion distributions to conform to the experimental observations regarding the variable extracellular ion content and membrane potential (Canals et al. 2005; Somjen 2001). Time evolving models compute the continuous variation of ions in and out of model cells as it rules the changes in  $V_{\text{m}}$  and hence channel conductances. A huge number of parameters required to model electrodiffusion, extracellular volume fraction, ion transport, and pumping is not essential for our steady-state approach. There is a likely contribution by electrochemical diffusion of ions in the zones separating SD-activated and -spared membranes. We have not implemented it as previous estimates show its little contribution to the generation of SD-associated  $V_{\text{o}}$  (Herreras and Somjen 1993a).

generation of SD-associated  $V_{\rm o}$  (Herreras and Somjen 1993a). The compartmental  $V_{\rm m}$ 's and  $I_{\rm m}$ 's were calculated using the GENESIS simulator (exponential Euler method: time step, 0.1 ms). The unit reproduces the known electrophysiological repertoire of pyramidal cells, as well as the contribution to the somatodendritic changes of  $V_{\rm m}$  and  $R_{\rm in}$  during SD.

SIMULATION OF THE AGGREGATE FIELD POTENTIAL. The spatial profile of  $V_{o}$  was calculated in a virtual interstitium along the dorsoventral extent of the CA1 pyramidal cell axis by means of a multineuronal model that uses a volumetric grid of current sources generated by the corresponding compartments of each single cell (Varona et al. 2000). We built a virtual aggregate of 17,299 morphologically identical neurons arranged in a realistic three-dimensional manner that represents a  $1 \times 1$  mm slab of tissue. The size of this aggregate ensures that the total contribution of the neurons outside this volume is <10% (Varona et al. 2000). Except when indicated, the somata of the units were arranged in four strata with a cell density of 64 neurons oriented in parallel in a 50  $\times$  50  $\mu m$  anterolateral lattice (Boss et al. 1987). The  $V_{0}$  was estimated in the virtual interstitium along a track of 16 "recording" points, 50  $\mu$ m apart (Fig. 1C). The V<sub>o</sub> at each recording point was evaluated as the weighted sum of all the compartmental  $I_{\rm m}$ 's

$$V_o(t) = \frac{1}{4\pi\sigma} \sum_{i=1}^{\text{cells comps}} \sum_{j=1}^{\text{comps}} \frac{I_{\text{m}_{ij}}(t)}{r_{ij}}$$

TABLE 1. Ionic concentrations (mM) during the different phases of SD

Phase	$[K^+]_o$	$[K^{-1}]_i$	[Na <sup>-</sup> ] <sub>i</sub>	[Na <sup>+</sup> ] <sub>o</sub>
Rest	3.5	133	20	140
SD main	40	125	35	90
SD late	30 (6)	125	35	90 (120)

At rest, all compartments are the same. During spreading depression (SD) main (SD running simultaneously through the apical and the basal dendrites), the values given are for ctive SD compartments only; all others are as in rest. During SD late (SD running through the apical dendrites only); the compartments that are no longer active still keep an intermediate ion oncentration (given in parentheses) to simulate their slow recovery.

where  $I_{m ij}$  is the current created by the *j*th compartment of neuron *i*, and  $r_{ij}$  is the distance from the recording point to the compartment. In previous work, we determined that tissue resistivity during SD changes inhomogeneously across strata (Makarova et al. 2008), although we set  $\sigma = 300 \ \Omega \times \text{cm}$  (López-Aguado et al. 2001) except in the SD-seized region where it was set fourfold greater (Makarova et al. 2008). This simplification admittedly introduces a bias that affects mostly to the relative  $V_o$  estimations between soma and dendritic regions.

SIMULATION OF SD-LIKE CONDITIONS. SD was mimicked by imposing experimentally guided transmembrane ion gradients (see Table 1) in all compartments within specific somatodendritic bands (see, e.g., Fig. 1B, left). The SD-like conditions were modeled by turning on a specific membrane conductance  $g_{SD}$ , which drives  $V_m$  and  $R_{in}$  of the selected dendritic compartments to the experimental SD values. Here we employed the conductance densities found before (Makarova et al. 2007) that range from 0 in resting compartments to  $100-1,000 \text{ pS/m}^2$ in SD activated zones at different time instants. The specific value in each case was estimated to match  $V_{\rm m}$  and  $R_{\rm in}$  values. The equilibrium potential was set to zero, and the Na<sup>+</sup>/K<sup>+</sup> permeability was 1. In some cases, this was modified so as to fit the local  $V_{\rm m}$  within the experimental values. At steady state, SD-like conditions were defined as  $-5 \le V_{\rm m} \le$ +5 mV and  $R_{\rm in}$  <5 M $\Omega$  [ $R_{\rm in}$  was measured by the voltage drop to injection of pulses of current as in Makarova et al. (2007)]. We tested numerous artificial and several realistic spatial distributions of SDaffected domains, so called main and late SD phases. In the former, SD simultaneously affects most of the apical and basal dendrites, while during the late phase it is restricted to a narrow apical band (Herreras and Somjen 1993a; Makarova et al. 2008).

 $V_{\rm o}$  FEEDBACK TO SINGLE-CELL MODEL. Typically, modeling environments (e.g., GENESIS) for single-cell simulations use the intracellular potential  $V_{\rm i}$  instead of the true membrane potential  $V_{\rm m}$ . In most conditions, this provides an accurate approximation, given that  $V_{\rm o}$  is negligible for computing channel conductances. However, during synchronous cell activation, as in evoked field potentials or SD, the  $V_{\rm o}$  can reach significant values and cannot be neglected. Earlier we have shown that its omission in the single-cell model introduces considerable error in simulation of SD (Makarova et al. 2007). In the present model, we explicitly introduced  $V_{\rm o}$  feedback in the calculation of channel conductances. In GENESIS, we used a workaround by introducing  $V_{\rm o}(t)$ , estimated in the 3D aggregate for each clock step, in the calculation of the  $E_{\rm Nernst}$  for the ion species employed to evaluate channel currents.

#### Experimental methods

We employed Sprague-Dawley rats (200-250 g) anesthetized with urethan (1.2 g/kg ip) and attached to a stereotaxic device. The surgical and stereotaxic procedures employed are described elsewhere (Canals et al. 2005). A stimulating electrode was placed in the ipsilateral CA3 for orthodromic activation of the CA1 pyramidal population. A vertical array of two pipettes, the tips of which were separated by 100  $\mu$ m, was used to record from one dendritic stratum while ejecting small drops (50-100 nl) of different salts into the other. A third pipette was placed caudally to elicit SD waves at a distant site by ejecting a similar drop of KCl (0.2-0.5 M). Injections in the apical tree elicit standard apical-leading SD waves, while injections in the basal tree elicit local basal-leading SD waves that turn into standard apical-leading waves at distant sites (Canals et al. 2005). After high-pass filtering (0-5 kHz band) and amplification, the electrical potentials were acquired (20-40 kHz acquisition rate, Digidata 1200, Axon Instruments, Burlingame, CA) and processed using Axotape and Axoscope software (Axon Instruments). All the experiments were performed in accordance with European Union guidelines (86/609/ EU) and Spanish regulations (BOE 67/8509-12 1988) regarding the use of laboratory animals.

RESULTS

# Distribution of transmembrane currents and potentials in single cells during SD-like conditions

The following results describe the electrical behavior of a single cell embedded in the virtual aggregate/extracellular space, i.e., subjected to the changes of  $V_{o}$  produced by the cooperative activity of their neighbor mates.

SUBCELLULAR GEOMETRY SHAPES MEMBRANE POTENTIALS AND CURRENTS. We performed numerical simulations with different spatial configurations of the SD affected domains. One to three active membrane domains of varying width and separation were assayed and the overall qualitative results are presented in Figs. 2 and 3. The effect of cell geometry on the  $V_{\rm m}$ 's and  $I_{\rm m}$ 's is shown in all compartments for two representative cases, separate SD activation of the basal (Fig. 2A) and apical (B) trees. In both cases, the membrane potential  $V_{\rm m}$  depolarized locally up to almost 0 mV in the active SD domains, while its value decreased toward the distal edges (see color-coded neurons). The  $I_{\rm m}$ 's have sandwich-like spatial distributions (i.e., strong active inward or negative currents flanked by shallow passive return outward currents: Fig. 2, right). In homogeneous linear conductors, passive currents decrease exponentially. However, units with a realistic pyramidal anatomy exhibited a notable deviation from this law, especially when active SD domains were in the basal tree near the soma (Fig. 2A). In this case, the  $I_{\rm m}$ 's display a peculiar asymmetric distribution with large amplitude in the outer basal border decreasing considerably toward the soma (Fig. 2A, *right*). This is due to the lower resistive load facilitating the spread of axial (internal) currents from the distal dendrites to the soma.  $I_{\rm m}$ 's are more symmetrical in the apical SD, although a small dip is still observed in the center of the active zone due to central cancellation (Fig. 2B, arrow b).

The higher axial resistance of the soma-to-basal junctions produced notable asymmetry in the spread of internal currents between the two dendritic trees, which transformed into different depolarizing effects of one active SD tree onto the other (cold color of basal dendrites in Fig. 2, A, vs. the "warmer" apical dendrites in B). With a basal SD, outward currents (blue compartments) were distributed all over the apical membranes, whereas they were negligible basally in conjunction with an apical SD. In both cases, the large surface area of the soma behaved as a shunt that returns an important fraction of the axial currents to the extracellular space (green circles in Fig. 2, *right*). As a result, the soma produces a steep fall in the  $V_{\rm m}$ profile, which was more pronounced in the direction of the basal dendrites. When the soma was also included in the active basal SD band, as occurred in some experiments (Canals et al. 2005), the apical  $V_{\rm m}$  profile was steeper (this simulation was superimposed as a red dashed line in Fig. 2A for comparison).

Note the behavior of the daughter (lateral) dendrites in the two flanks of the SD activated apical domain (arrows "a" in Fig. 2B). Those in the direction of the soma drain less outward current than their parent compartments, whereas those toward the distal tuft drain more. As expected, the daughter dendrites in passive zones displayed a smaller depolarization than their parental apical shaft. However, the opposite behavior was observed in SD active zones (arrow "c" in Fig. 2B).



FIG. 2. Effect of cell geometry on the spatial distribution of  $V_{\rm m}$  and  $I_{\rm m}$ . SD activation of narrow portions of the basal (A) and apical (B) dendritic trees leads to significantly different  $V_{\rm m}$  and  $I_{\rm m}$  distributions (active SD zones in this and the next figures are horizontal dashed markings of the abscissal scales and the shaded areas on the colored neurons). Each dot represents the value of a cell compartment. Red dots correspond to the main apical shaft and black dots to thinner dendrites in the basal and apical trees. The neuronal membrane is depolarized close to 0 mV at the active sites of SD, and  $V_{\rm m}$  decays toward the edges at a varying rate according to the cell geometry. Transmembrane currents flow inwards at the activated SD membranes and outwards in the flanking domains. The soma (green dot) produces a strong asymmetric decay of the  $V_{\rm m}$ , and it also drains most of the current, producing asymmetric distortion on the internal distribution of currents and potentials (e.g., arrows a). A dip in the inward  $I_m$  (arrow b) indicates partial central cancellation of axial and transmembrane currents. The red dashed line corresponds to another example in which SD activation extended to include the soma (see further details in the main text). Calibration bars for color coded neurons in this and the next figure (cold to warm pitch): -65 to 0 mV for  $V_{\rm m}$  and -1.5 to 1.5 nA for  $I_{\rm m}$ .

CO-ACTIVATED DOMAINS PROMOTE ISOPOTENTIALITY. To study the interaction between two activated dendritic domains of SD, we performed multiple simulations in which the domain configuration was changed. These can be summarized in two representative configurations: nonoverlapping apical and basal SD and overlapping SD partially affecting basal, somatic, and apical domains. In the nonoverlapping case, the spatial profile of  $V_{\rm m}$  was bimodal, with two maxima in the active SD zones and a less depolarized middle region. Indeed the wider the activated regions, the stronger the depolarization of the interposed region. Complete isopotentiality was attained when the activated SD domains overlapped to form a single active SD domain (Figs. 3B and 4).

Co-activated basal and apical domains had an asymmetric effect on one another. While basal inward currents were barely modified by the presence of an apical active domain, those on the apical side were reduced by a basal SD (arrow and blue line in Fig. 3A, *right*). Generally, the membrane domains between the two active SD regions carried a net outward current. However, when the entire region was activated by the SD (i.e., only 1 extended SD generator exists), the interior compartments became completely isopotential and the  $I_m$ 's fell close to zero (Fig. 3B, *right*). Thus the middle region (including the somata) does not behave as a current source for the extracellular space and only the outer flanks contribute to the net outward current.

In general, the interaction between two separated SD membrane generators not only changed the spatial distribution of  $I_{\rm m}$ along the neuron anatomy but also reduced the net inward  $I_{\rm m}$ .

# Building the macroscopic $V_o$ from the transmembrane currents in single cells

SUBCELLULAR INTERACTIONS HAVE A STRONG IMPACT ON THE AG-GREGATE  $V_{o}$ . The aforementioned changes in the spatial distribution of the  $I_{\rm m}$  in single cells were tested for their impact on the aggregate  $V_{o}$ . We first explored the influence of the size of the active SD membrane generators using a population of simplified single-cell units (see METHODS). This allows detaching the extension of activated subcellular domain from the specific macroscopic effects due to the particular geometry of pyramidal cells. The simulation settings (3D aggregate, SD conditions, etc.) were the same as for calculations made with the branched neuron.

SD activation in the ideal homogeneous geometry produced the expected symmetric bell-shaped  $V_{\rm m}$  depolarization and a sandwich-like distribution of inward/outward currents (Fig. 4). The greater the SD activated surface, the deeper the depression of the inward currents in the center, which approached zero for activated extensions >400  $\mu$ m. This central region of low  $R_{\rm m}$ , strong depolarization, and a null  $I_{\rm m}$  expanded outwards from the center to finally cover the entire array of conductors when



FIG. 3. Activation of extended membrane domains leads to the central cancellation of currents. A: simultaneous SD activation of 2 separate membrane loci produces spatial bimodal distribution of  $V_{\rm m}$  and slight changes in the distribution of compartmental  $I_{\rm m}$ 's (compare with apical only SD activation in Fig. 3B, blue line). B: SD activation of extended neuron domains produces isopotentiality throughout most of the shunted membranes and a strong central cancellation of axial and transmembrane currents. The inward  $I_{\rm m}$  is only maintained at the outer rims of SD activated membranes corresponding with the presence of longitudinal gradients of  $V_{\rm m}$ . See further explanation in the text.

SD activation was completely homogeneous as would be expected for complete isopotentiality. The reduction of total inward current by central cancellation compensated for the expected increase due to wider activated domains (Fig. 4, *inset*). Consistently,  $V_o$  was also smaller for large domains. Thus central cancellation imposed a high limit to the maximum amount of current that a single neuron may inject to the extracellular space.

Besides the amount of current, its spatial distribution is also a relevant factor for  $V_{\rm o}$  generation. Indeed although the estimated  $V_{\rm o}$  showed a spatial bell-shaped distribution mirroring the profile of the  $V_{\rm m}$  values distributed among the array of cells, the margins were sharper and aligned with the spatial distribution of  $I_{\rm m}$  in single cells from which it actually arose. The spread of currents in the volume conductor smoothed the spatial profile of  $V_{\rm o}$ . Note that a positive  $V_{\rm o}$  developed at both edges with a very small amplitude despite the moderate size of the outward currents (see modifications to this pattern by introducing a large soma-like compartment in Fig. S1 of the supplementary material).

Importantly,  $V_{\rm o}$  reached a steady plateau for widely spread activations that spanned the region where  $I_{\rm m}$  in single cells was zero as observed for experimental SD in its wider spatial coverage. We performed numerical simulations that demonstrated that this is the expected field distribution for two separated laminar dipoles of opposite polarity (see Supplementary Fig. S2) as one may intuitively envisage the spatial clustering of the marginal neuronal domains that produce current in the flanks of the null center domain.

EXTRACELLULAR POTENTIAL DURING HIPPOCAMPAL SD. Let us now study the particular configurations of SD activated membranes that fit the experimental spatial configurations of hippocampal negative  $V_{0}$  (see supplementary material and Fig. S4 for the specific SD spatial details in this region). For these simulations, we used the model unit with realistic branched geometry. SD activation of one dendritic zone in all the cells of the simulated CA1 aggregate (Fig. 5A, MOD) created a large negative  $V_{0}$  band that was centered in the region containing the activated neural domain, and it was surrounded by shallow positive fields (asterisk) extending toward the distal borders of the neuron palisade. This configuration mimicked the experimental situation when SD runs in one dendritic tree only (e.g., basal in Fig. 5A, EXP). The magnitude of  $V_0$  depended on several parameters. Indeed for a tissue resistivity fourfold the control value (Makarova et al. 2008), with conductance densities ranging  $10^2-10^3$  mS/cm<sup>2</sup> (Makarova et al. 2007) and activated dendritic domains varying from 100 to 450  $\mu$ m, we obtained  $V_{0}$  maximum values ranging from 20 to 38 mV. The inclusion of the soma in the zone activated by the SD produced a notable increase in  $V_{o}$  (Fig. 5A, MOD), in agreement with the experimental data (Makarova et al. 2008). As expected from the behavior of transmembrane currents in single cells, activation of narrow dendritic bands yielded large bell-shaped  $V_{0}$ profiles, while wider activation domains produced wider and



FIG. 4. Spatial correspondence of unitary and aggregate electrical features for different extensions of SD activated membrane domains. The top horizontal bars delimit the size of the active SD membrane for 7 simulations made using an aggregate of parallel simplified units with the flat geometry sketched at the top. The wider the SD activated membrane domain, the stronger the central cancellation of inward currents. Note that the maximum  $V_o$  produced by the aggregate increased and then decreased before reaching a plateau for wider SD activation. At the same time, the total net inward current in single units first increased and then decreased (*inset*) and split into 2 separate bands at the margins of activated membranes. Volume spread of currents smoothed the spatial  $V_o$  profiles as compared with the split distribution of  $I_m$  in single cells.

smaller profiles with flat (plateau like) centers. This profile was particularly evident at the beginning of a standard experimental SD in the CA1 when the SD activates a large domain from the basal to apical dendrites (Fig. 5*B*). Such spatial plateaus of negative  $V_{\rm o}$  (profile *a* in Fig. 5*B*, MOD) roughly coincided with the dendritic domains of zero  $I_{\rm m}$  (see, e.g., Fig. 3*B*).

Experimental SD waves running in one dendritic tree are very valuable to analyze the passive return currents. These currents produce distinct positive fields with a distribution that arose from the geometrical properties of the neuron (Fig. 5, Aand C). Significantly, with narrow bands of activation in only one dendritic domain positive, fields developed in the dendritic tree opposite the activated side (asterisks in Figs. 5A, MOD) while they barely reached zero in the distal portion on its own side. This effect arose through a combination of geometrical factors, such as the presence of a large interposed soma, or due to the distance from the active band to the soma and to the outer border on that side (see Supplementary Fig. S1). This type of small positive field is larger in the apical than in the basal tree due to the facilitated spread of axial currents from the basal to the apical tree given the smaller resistance of the somato-apical junctions.

When the basal SD appeared transiently during an apical SD (Fig. 5C), it produced a large, distinct positive-going  $V_0$  on the ongoing apical negativity. This was observed in both experiments and models. At the same time, there was a reduction in the maximum negative  $V_{0}$  associated to the apical SD, such that the  $V_{0}$  equalized throughout the simultaneously activated membrane loci (compare spatial profiles in Fig. 5C, right). This effect was produced by the mutual cancellation of opposing axial currents from the two separate membrane shunts; this is much more effective than the passive drain of currents across adjacent membranes with an intact  $R_{\rm m}$ . In experiments, the amplitude of the positively orientated  $V_0$  humps was very variable ( $\sim 2-14$  mV), the maximum being roughly the same as the negative  $V_{0}$  reached by the transient generator that it equalized. Two observations should be noted here, the selective decrease of  $V_0$  on the apical side and a negative "fill-up" effect in the interposed domains, which finally configured a single negative plateau extending throughout the entire active region.

The role of the axon on  $V_{o}$  production was negligible (see additional simulations in supplementary material and Fig. S3).

POPULATION ARCHITECTURE: THE TIGHTLY PACKED ARRANGEMENT OF CELLS BOOSTS THE SD ASSOCIATED  $V_0$  NEGATIVITY. We assessed whether the highly ordered architecture of principal cells in the hippocampus may explain the amplitude of the SD associated  $V_{\rm o}$  in the CA1, which is larger than in other brain regions. We ran simulations with a population of simplified neurons in which the cell somata were either tightly packed within a 50  $\mu$ m thick layer (as in the real CA1) or scattered through a 400  $\mu$ m wide band (as in some cortical layers). For comparison, we preserved the same number of units and did not introduce a heterogeneous spatial profile of the extracellular resistivity. A narrow apical band (100  $\mu$ m) of SD activation was employed to lessen the impact of  $I_{\rm m}$  cancellation within single cells. Accordingly, the spatial profile of  $V_{\rm o}$  became smoother, and its maximum fell about 50% in the scattered versus the tight arrangement (Fig. 6). In addition, flanking positive fields were proportionally less affected than negative ones. Thus the average volume of currents from units that individually undergo similar activation at least in part accounted for the electrophysiological differences reported between the cortex and hippocampus.

#### Testing theory experimentally

The results of the simulations indicated that the primary mechanism in the generation of sustained extracellular potentials during SD was the polarization of different domains in the neuron. Accordingly, one might expect an increase in the isopotential area within single cells if the outer limits of the SD-activated domain extended (Fig. 3B). In turn, the decrease in  $I_{\rm m}$  and the consequent aggregate negative  $V_{\rm o}$  should reduce its magnitude. Thus this inference was tested experimentally by injecting small drops of depolarizing potassium salts (60 mM KCl +90 mM NaCl) through a pipette at sites and time instants during the evolution of the SD within the lateral wall of the SD negativity (i.e., where dendritic domains sustain depolarization gradients: Fig. 7*A*). The variations in  $V_o$  at adjacent sites were recorded with another pipette located 100  $\mu$ m below. When potassium was injected at an SD moment when the  $V_o$  was returning to the baseline, a negative dip was recorded in both electrodes (Fig. 7*A*, *a*, *c*, *f*, and *g*). However, when the site of the injecting pipette was already in the recovering zones (returning to 0 mV) but the recording pipette was still within zones of steady negativity, the dip in the latter was positive (Fig. 7*A*, *b*, *d*, *e*, and *h*–*l*).

This experimental widening of the depolarized dendritic domains was also assayed by the model (Fig. 7B). We chose an



apical band of active SD domains (blue rectangle) and simulated  $K^+$  injections by introducing an extracellular environment of increased  $K^+$  at different loci relative to the SD dendritic band (cyan rectangles). As can be seen, potassium enriched extracellular domains always produce a local negative dip (arrows in Fig. 7*B*, *a*–*c*), even within the SD activated domains that had lower potassium (Fig. 7*B*, *f*). However, SD activated domains adjacent to potassium enriched zones displayed a dip toward positive (e.g., black and red traces in *a*–*c*). Similar positively orientated dips were also recorded in SD active domains when potassium induced depolarization was aimed somewhat further away, and as such the largest positive humps were produced when the depolarization was distal in the opposite tree. Thus the experimental observations confirmed the predictions of the model.

#### DISCUSSION

The computational model of SD presented here aimed to simulate the subcellular mechanisms underlying the experimentally observed giant negative extracellular potential  $V_{o}$ . This model enables the transmembrane currents to be estimated across the subcellular anatomy of single cells (see *Distribution of transmembrane currents and potentials in single cells during SD-like conditions*) as well as their sum in the extracellular volume (see *Building the macroscopic V<sub>o</sub> from the transmembrane currents in single cells*). The model neurons were subjected to SD, as simulated by experimentally demonstrated differential polarization, the membrane shunting of discrete domains, and local changes in ion gradients. Analysis of the

FIG. 5. Simulated dynamics of the extracellular potential reproducing common experimental cases in the CA1. In the top left corner of each section there is a space time diagram of the progress of the  $V_0$  shift included to illustrate the experimental cases simulated (right). Dummy neurons are filled in a gray scale to indicate varying subcellular depolarization (darker tones mean larger depolarization), according to experiments. The standard case is B, while A and C are less frequent (the order is based on increasing complexity). Basal and apical SD waves are shadowed differently. Vertical dotted lines are for time reference with actual recordings of  $V_{o}$  in the soma (black) and apical (gray) layers. Horizontal dashed lines outline the pyramidal cell layer (not to scale). In the simulations (MOD, right) space and time plots are shown in the upper and lower panels, respectively. Asterisks in the spatial plots mark positive V<sub>o</sub> residues, similarly to the gray (negative) and black areas (positive) on top of  $V_0$  recordings. These are produced by strong SD activity in distant cell domains (see text). A: (EXP) SD invades 1 dendritic tree at a time, first the basal and then the apical tree. (MOD) Simulations of basal SD for 2 slightly different extensions of the SD activated domains. Upper panel shows two spatial maps of V<sub>o</sub> corresponding to the SD activated domains marked by bars in the abscissa. The small arrows indicate the recording position for the corresponding temporal displays in the bottom panel. Arrowheads a and b near the steady state mark the time instants used to draw the spatial plots above. Note the bell-shaped  $V_{o}$  negativity and the positive potential in the dendritic tree opposite to the SD active side (black areas in both experimental and model traces). B: standard SD wave with a shorter component in the basal tree. The reduced  $V_0$  in the initial phase is due to the simultaneous activation of SD through an extended portion of the cell covering most of the basal and apical domains, and the soma layer. After the SD subsided in the basal an soma layers, the negative  $V_0$  reaches its full amplitude due to the partial relief from the internal cancellation of currents in single cells. Note that apical SDs leave a small negative  $V_0$  in the soma layer (gray areas) that reverses to positive at the basal sites (asterisk). C: transient basal SD during an ongoing apical SD. Note the reduction of the ongoing negative  $V_{o}$  related to the apical SD during basal SD (black areas) in both the model (a vs. b) and experimental results. Vertical calibration: 10 mV; horizontal calibration: 10 s (EXP) and 0.1 s (MOD)



FIG. 6. Orderly arranged cells boost the  $V_o$  during SD. A 100  $\mu$ m apical dendritic band was SD activated on each cell in an aggregate of 17,299 parallel units arranged with a vertical dispersion of 50  $\mu$ m (CA1-like, black) or 400  $\mu$ m (cortex-like, gray). The  $V_o$  is uncorrected for extracellular resistivity as we have no measurement for the latter and hence, it is given in arbitrary units. The extracellular clustering of inward currents boosts the  $V_o$ . Note the smoother profile and the disproportionate reduction of flanking positive fields in the spatially scattered population.

SD model shows that sustained loops of transmembrane currents within individual neurons can explain the spatial and temporal state of single cells, as well as the spatiotemporal evolution of the aggregate  $V_o$  in the hippocampal CA1 region. We also show that the results from the model are in agreement with the experimental data (see *Testing theory experimentally*).

#### Methodological considerations

The present model uses a steady-state approach that yields numerical solutions at specific time points during SD evolution. Because each time point is defined by a particular spatial distribution of the  $R_{in}$  and  $V_m$  over the subcellular anatomy of a pyramidal cell, the model cannot provide a detailed dynamic description of extraneuronal variables. Rather than being a drawback, this approach implies an important reduction of the parametric space (Makarova et al. 2007) while it remains efficient in estimating compartmental  $I_m$ 's, which is the main component required to reconstruct the macroscopic  $V_o$ .

An important improvement in the model used here is the explicit use of the dynamics of  $V_0$  in calculation of the compartmental transmembrane currents in the single-cell model. This feature is not essential to reproduce fast transient fields, such as population spikes (Varona et al. 2000), because their time scales are much shorter than the time constants of most V-dependent channels. However, the opposite occurs during self-sustaining long-lasting events (e.g., during SD). Hence neglecting the contribution of  $V_0$  to the transmembrane potential  $V_m$  modifies the magnitude and can even change the sign of the  $I_m$ . To assess this, we repeated some simulations using the intracellular potential  $V_i$  as the transmembrane potential instead of  $V_m$ . Such simulations yielded a significantly lower  $V_0$  ( $\leq 30\%$ ), confirming the need for  $V_0$  consideration in the single-cell model.

To save computation time, a simplified spatial profile of tissue resistivity is employed here in which the soma and dendritic layers were established as homogeneous based on our own experimental evidence (Makarova et al. 2008). Admittedly, this simplification led to quantitative errors in the  $V_{\rm o}$  estimated for this layer when the soma has a significant current flow. However, this is not a major source of error for hippocampal variants of SD because the soma had either a negligible current contribution when the SD spanned most of the neuron or it contributed passive (small) currents during apical SD alone (Makarova et al. 2008).

In the present simulations, we have not implemented anions. Chloride is important for the velocity of SD propagation and the tissue susceptibility to initiate the reaction (Phillips and Nicholson 1979), but it does not contribute significantly to its amplitude (Müller 2000). In any case, what is relevant for  $V_{\rm o}$  production is the total current across membranes, whichever the channel carrier. Because  $V_{\rm m}$  and  $R_{\rm in}$  are imposed by experimental results, a possible effect caused by the omission of chloride would only require a change in the relative permeability utilized for other ion species to maintain these values.

#### Loops of current and subcellular geometry

It is well known that current spreads within and outside branched conductors during transient activation (Jack et al. 1975; Lorente de Nó 1947). Although SD develops unusually sustained fields, we show that these also adhere to the same principles of core conductor and field theories, albeit in a steady-state case. After an initial period of stabilization, when  $V_{\rm m}$  is governed by the strict kinetics of V-dependent channels, a plateau is reached the magnitude of which depends on the local transmembrane ion gradient and the relative membrane permeabilities as occurs for neurons at rest. The main difference under SD is that neurons are subject to different extracellular ion concentrations across their anatomy (Herreras and Somjen 1993a), which sets a different  $V_{\rm m}$  (Canals et al. 2005) in different regions of the same conductors. The longitudinal gradients of polarization created initiate axial currents from depolarized to more polarized compartments. These axial currents are contiguous with in- and outward currents in the depolarized and flanking domains, respectively (i.e., extracellular sinks and sources closing the current loops).

In linear homogeneous conductors, current loops are distributed symmetrically, while heterogeneous and branched structures notably modify the internal and transmembrane flow of currents. The most influential factor is a large soma connecting two branched dendritic trees. The soma acts as the main source of current for dendritic membranes, and it moves the distribution of return currents away from the expected exponential decay. In customary evoked field excitatory postsynaptic potentials (fEPSPs), the soma layer produces strong sources and positive fields that are passive to inward currents in dendrites (Herreras 1990) and that do not occur during a standard SD. A strong surge of inward current in dendrites and outward current in the cell body layer was reported in a previous current source density (CSD) study (Wadman et al. 1992). In general, our CSD study (Makarova et al. 2008) and the present model agree with these findings except in some of the spatial details derived from the higher resolution obtained in our work. Specifically, the dendritic sink (inward current) is restricted to the outer domains while the outward current at the soma level only occurs during the late SD phase, when it runs in the apical tree. The lack of the outward current at the soma in the main SD phase is explained by the fact that this compartment is isopotential with surrounding basal and apical compartments, and hence, it falls under the effect of central single-cell cancellation.

We also noted that the different size of the basal and apical somatodendritic junctions forces the soma to act as a unidirectional filter in the spread of axial currents between dendritic trees. This has important macroscopic consequences as it produces larger positive fields in the apical than in the basal tree for SD waves running on the opposite side as also seen experimentally. This difference could not be explained by nonneuronal mechanisms of  $V_{\rm o}$  generation such as electrodiffusion in the extracellular space (Almeida et al. 2004). Indeed this geometrical factor might underlie the experimental observation that the initiation of SD in the basal dendrites is typically followed by subsequent apical activation, while the converse is not true (Canals et al. 2005; Herreras and Somjen 1993a,b).

When active domains extend through very large portions of the neuron, as in the early phase when SD occupies most of the basal and apical dendrites, central cancellation is strong enough to annihilate the  $I_m$  along most of the active membranes despite the strong steady depolarization. Isopotentiality sets up in these do-



mains, nullifying axial currents everywhere except in narrow bands at the borders. Accordingly, these become the only cell domains producing sinks of current in the extracellular space the return currents of which pass through the less depolarized distal tips of dendrites as also confirmed experimentally (Makarova et al. 2008). The fact that  $V_{o}$  is steadily negative in the central regions of null transmembrane current may appear counterintuitive, but nevertheless it is the result expected for two separated laminar dipoles with negative poles facing each other (see Supplemental Fig. S2). Additional observations indicate that such a peculiar field distribution is generated by complete central cancellation in a single core conductor that becomes then functionally split in two as opposed to two separated core conductors. Thus the decrease in the apical negativity by the transient eruption of a basal SD is only possible if both belong to different domains of the same core conductor. It can be shown that if basal and apical SDs were independent of one another (i.e., generated by different spatially separated core conductors), variations in one would barely affect the other, and the sum of the fields in the interposed regions would not normally develop a spatially equalized steady negative  $V_0$ . Furthermore, the strong decrease in  $V_{0}$  of the ongoing apical SD during a basal outbreak of SD is not accompanied by extracellular ion changes (cf. in Fig. 4 of Herreras and Somjen 1993a). Hence this effect would appear to be caused by internal cancellation of local currents on the activation of a generator in another portion of the cell rather than local changes in activated membranes. Indeed, the late apical-only phase in experiments actually results from the early termination of SD in the somato-basal zone. This narrowing of the SD affected domains diminishes central cancellation in apical dendrites that is replaced by a large narrow sink.

# Loops of current in neurons versus alternative mechanisms for $V_o$ generation

The mechanism of  $V_{o}$  generation during SD studied here was first proposed by Leão (1951) himself and then by others, and it adheres to the standard postulates of core conductor and field

Experimental assessment of model predictions: extending the area FIG. 7. of depolarized membrane domains causes a reduction in the ongoing  $V_{0}$ negativity in adjacent domains. A: space time diagram of the  $V_0$  shift in experiments. An array of 2 pipettes served to inject potassium salts locally through 1 pipette (black) while recording at that site and at an adjacent site 100  $\mu$ m below (blue). Depolarizing salt injections were applied at specific instants and sites of advancing SD waves, while the  $V_{0}$  recorded by the lower pipette was either full size or already recovering (1-4 pairs of dots in the scheme). Potassium injections at instants when both sites are repolarizing (1) produce negative dips in  $V_{0}$  at both sites, while positive dips are found only in noninjected sites when these are still in fully depolarized regions (4). Red traces in the inset correspond to noninjected waves for comparison (see the main text for further explanation). B: model replication of the above experimental design. A narrow band of SD-activated membranes (light blue) was employed and small domains were transiently depolarized (cyan) at different sites along the model pyramidal units. Each configuration in the upper scheme is exemplified by a single dummy neuron but actually, it represents the settings for the entire population. For clarity only a few recordings in and close to the SD-activated membrane are plotted (colored plots correspond to the virtual "electrode positions" 9–13). Some injection sites are marked by small arrows in the traces. Settings b,c reproduce the experimental configuration in part A (see the positive dips in electrodes 10-11). Similar results are obtained in settings a, e, and g for injections on the other side. In setting f, potassium injection adds negativity, as occurs in active SD membranes.

theories for the generation of neuronal electrical activity. The popularity of this hypothesis has suffered ups and downs, and the incomplete understanding of the subcellular electrogenesis of neurons during SD made it vulnerable to speculation raised by rough analogy of SD to other mass phenomena.

The much smaller  $V_{0}$  shifts recorded during repetitive activation or epileptic seizures are usually attributed to spatially distributed currents flowing through the electrotonic quasisyncytial glial network that return through the interstitial space (Dietzel et al. 1989; Somjen 1973). For some time, many authors considered this mechanism to underlie the large  $V_{0}$ recorded during SD and hypoxia. However, it was shown that the selective disruption of glial function not only decreases  $V_{0}$ during SD, but it also boosts some of its features (Largo et al. 1996, 1997). Indeed dissociation of glial propagated calcium waves and SD has also been reported (Peters et al. 2003). The participation of glia in even slower and smaller V<sub>o</sub> shifts extending beyond the characteristic negativity cannot be ruled out. Also in the retina, the parallel arrangement of elongated glial Müller cells may provide a similar mechanism to that proposed here for neurons.

Another possible mechanism for the generation of sustained  $V_{\rm o}$  is based on the buildup of ion gradients in the interstitium. We previously calculated that due to this mechanism, the zone invaded by SD may be 4.6 mV more negative than the surrounding tissue (Herreras and Somjen 1993a). We speculated that this value could climb to 72 mV if potassium were the only ion species, which might occur if swollen cells at the front of the wave had completely occluded the interstitium. However, there is no experimental support for such a possibility to date.

Electrodiffusion of ions has typically been considered as a mechanism for SD propagation that was implemented in many previous SD models, while the generation of  $V_0$  has only rarely been included. Only one model (Almeida et al. 2004) predicted negative values slightly above 10 mV, considered by the authors sufficient to explain the cortical SD. However, even this value was probably overestimated. Other experimental results also argue against a significant contribution of electrodiffusion. Although the changes of interstitial ion concentrations during SD are similar in different brain regions,  $V_0$  in the in vivo CA1 is two to four times larger (Herreras and Somjen 1993a). We show here that a slight dispersion of neurons in the volume conductor may well account for these quantitative differences. In addition, positive fields raised by passive currents are reduced much less than negative fields as explained by the stronger volume averaging of the narrow active sinks when compared with the wider passive sources. Curiously, smaller negative  $V_{0}$  and proportionally large positive fields are observed in the cortex and retina where neuron conductors are not as tightly packed as in the hippocampus. Finally, although extracellular potassium level gradually declines from its peak during the sustained  $V_0$  shift of SD in any brain region, the negative  $V_0$ follows different trend: decreasing in the cortex, retina, and cerebellum, while increasing in the hippocampal CA1 (do Carmo and Martins-Ferreira 1984; Herreras and Somjen 1993a). To our knowledge, there is no evidence of mechanistic differences in ion diffusion in the interstitium, in the clearance capacity of astroglia, or in the membrane selectivity for potassium in the cortex or the hippocampus. Thus the most likely explanation for the difference in  $V_{0}$  production is the monolayered architecture of the hippocampal CA1 as opposed to the tangled neuropil in other regions where processes from different core conductors mix and cancel one another out, thereby reducing the  $V_{0}$  magnitude.

### Final remarks

Our analysis has shown that the unitary and macroscopic electrical features of SD can be explained by the selective depolarization of neuron domains during SD. To our knowledge, this is the first time that core conductor and field theories have been linked to reproduce sustained electrical signals in the brain. Of practical interest, variations in macroscopic potentials are not only related to changes in the intensity of activation, or in the number of contributing units, but they may also reflect changes in the electrical interactions between subcellular domains highly dependent on their particular geometry.

In the clinical literature, SD is rather simplistically viewed as a potassium wave moving in the neuropil. Both experiments and models support the main contribution of a number of membrane channels, some even unknown, indicating that multiple membrane targets should be considered as simultaneous targets in clinical trials. Indeed it is becoming increasingly relevant to monitor SD or SD-like depolarization in cerebrovascular accidents to diagnose the extent of subsequent neuron damage (Strong et al. 2007). Hence be able to precisely interpret its most characteristic feature should help in designing suitable interventions.

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#### DISCLOSURES

No conflicts of interest are declared by the authors.

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