Role of Dendritic Spines in Action Potential Backpropagation: A Numerical Simulation Study

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Tsay, David and Rafael Yuste. Role of dendritic spines in action potential backpropagation: a numerical simulation study. J Neurophysiol 88: 2834–2845, 2002; 10.1152/jn.00781.2001. Two remarkable aspects of pyramidal neurons are their complex dendritic morphologies and the abundant presence of spines, small structures that are the sites of excitatory input. Although the channel properties of the dendritic shaft membrane have been experimentally probed, the influence of spine properties in dendritic signaling and action potential propagation remains unclear. To explore this we have performed multi-compartmental numerical simulations investigating the degree of consistency between experimental data on dendritic channel densities and backpropagation behavior, as well as the necessity and degree of influence of excitable spines. Our results indicate that measured densities of Na⁺ channels in dendritic shafts cannot support effective backpropagation observed in apical dendrites due to supra-threshold inactivation. We demonstrate as a potential solution that Na⁺ channels in spines at higher densities than those measured in the dendritic shaft can support extensive backpropagation. In addition, clustering of Na⁺ channels in spines appears to enhance their effect due to their unique morphology. Finally, we show that changes in spine morphology significantly influence backpropagation efficacy. These results suggest that, by clustering sodium channels, spines may serve to control backpropagation.

INTRODUCTION

Pyramidal neurons have been one of the most studied cell types due to their key excitatory role in the cortex (Feldman 1984; Ramón y Cajal 1904). Recent studies have shown that the dendritic membrane of pyramidal neurons is active and capable of supporting the propagation of action potentials, elicited from various initiation zones (Larkum et al. 2001). Sodium action potentials (Na⁺ APs) initiated at the axon can “backpropagate” into the apical dendritic tree (Buzsáki and Kandel 1998; Stuart and Sakmann 1994;Stutt et al. 1997a) while distal dendritic sodium-calcium (Na⁺-Ca²⁺) spikes are able to forward-propagate toward the cell body (Helmchen et al. 1999; Schiller et al. 1997). Dendritic signal propagation is supported by dendritic Na⁺ (Huguenard et al. 1989;Magee and Johnston 1995) and K⁺ channels (Bekkers 2000b; Johnston et al. 2000; Korngreen and Sakmann 2000). Indeed the active properties of the dendrite may be the basis of functional compartmentalization of the neuron (Johnston et al. 1996; Pinsky and Rinzel 1994; Yuste and Tank 1996; Yuste et al. 1994).

Of the modes of signal propagation in dendrites from pyramidal neurons, backpropagation has been the most extensively characterized (Stuart et al. 1997b). This retrograde signal effectively invades the apical dendritic tree and its spines and is thought to play an important role in synaptic plasticity (Linden 1999; Magee and Johnston 1997; Markram et al. 1997; Yuste and Denk 1995). Modulation of AP backpropagation could be achieved by changes in dendritic morphology (Mainen and Sejnowski 1996; Stuart et al. 1997b) and by regulation of voltage-gated channel density and their spatial distribution (Johnston et al. 1999, 2000). In addition, recent studies have shown that backpropagation can be modulated by neurotransmitters and second messenger systems via alterations in channel kinetics (Hoffman and Johnston 1999;Johnston et al. 1999) and may be dependent on the level of synaptic activity (Pan and Colbert 2001).

A remarkable aspect of dendritic morphology in pyramidal neurons is the abundant presence of spines, small mushroom-like structures that decorate dendritic branches and are the primary sites of excitatory input. Spines, which attach to the dendritic shaft by a short neck, have densities ≤10 pm and can make up >50% of the total dendritic membrane. Although the miniature spine head (<1 μm in diameter) has yet to be probed directly by electrical techniques, theoretical (Kawato and Tsukahara 1984;Koch and Poggio 1983;Segev and Rall 1995), imaging (Sabatini and Svoboda 2000; Yuste and Denk 1995), and immunocytochemical studies (Caldwell et al. 2000) have suggested that the spine is endowed with voltage-gated channels. The overall influence of spine membrane properties on dendritic function has been suggested to be quite significant (Baer and Rinzell 1991; Segev and Rall 1998). Recently, measurements of membrane currents from dendritic patches have become available (reviewed in Johnston et al. 1996). This has prompted our study, using numerical simulations, on the relative importance of excitable spines and their necessity in dendritic signal propagation. In particular, we investigated the degree of agreement between measured channel parameters in dendritic shafts and the well-documented backpropagation. We have then explored to what extent spine channels are needed to account for the measured data, by comparing simulations of backpropagation with excitable spines to those without them. We find that dendritic shaft channel densities and kinetics found experimentally are unable to support effective backpropagation. At the same time we demonstrate that excitable...
spines may be a necessary and efficient factor in regulation of backpropagation, and that clustering in the unique spine morphology provides greater sodium channel activation. Our conclusions are relevant to the role of synaptic activity in regulating dendritic signaling and the processing strategies of spiny neurons.

**METHODS**

**Morphological reconstructions**

A detailed three-dimensional reconstruction of a layer 5 pyramidal cell was made from a postnatal day 15 C57/B6 mouse primary visual cortex (Kozloski et al. 2001) (Fig. 1A). The neuron was identified because it projected to the superior colliculus, as revealed by its retrograde labeling with fluorescence latex microspheres. Three-dimensional digital reconstruction was performed using a 60×/1.4 NA oil objective using Neurolucida software (Microbrightfield, Colchester, VT) on an Olympus IX70 (Melville, NY) microscope. All dendritic (apical and basal) and axonal compartments were carefully reconstructed. Spines were modeled explicitly, each with 1-μm head diameter, 1-μm neck length, and 0.2-μm neck diameter (Harris and Kater 1984). Spine neck resistances were calculated using the following equation

$$R_s = \frac{(4 \cdot R_c \cdot L)}{(\pi \cdot d_n^2)}$$

where $R_s$ is the axial resistance, $L$ is the length of the neck, and $d_n$ is the diameter of the neck.

Each spine consisted of a head and neck compartment; the distal end of the neck was connected to the central head, while the proximal end was connected to the dendritic shaft. Spine density in the apical end was connected to the dendritic shaft. Spine density in the apical end of the neck was connected to the central head, while the proximal end was connected to the dendritic shaft. Spine density in the apical end was connected to the central head, while the proximal end was connected to the dendritic shaft. Spine density in the apical end was connected to the central head, while the proximal end was connected to the dendritic shaft. Spine density in the apical end was connected to the central head, while the proximal end was connected to the dendritic shaft. Spine density in the apical end was connected to the central head, while the proximal end was connected to the dendritic shaft. A total of 3,034 spines were inserted, and they contributed approximately 50% of the total dendritic membrane. In our model, we did not reduce the computational complexity presented by the inclusion of spines by use of any approximation methods (Baer and Rinzel 1991; Stratford et al. 1989) since computational power of processors recently have been sufficient to handle the number of equations presented.

**Numerical simulations**

Numerical simulations were performed using NEURON (http://www.neuron.yale.edu/neuron/papers/97/nctox.htm; Hines and Carnevale 1997). Numerical calculations were needed to address the behavior of active channels, since no robust analytical method exists for addressing membranes with nonlinear and/or nonuniform properties (London et al. 1999). Passive cable properties were chosen in accordance with studies on pyramidal cells (Magee and Johnston 1995; Stuart and Spruston 1998): $R_m = 160 \, \text{k} \Omega \cdot \text{cm}^2$, $C_m = 0.8 \, \mu \text{F/} \text{cm}^2$, and $R_a = 80 \, \Omega/\text{cm}$. Simulations were performed at a temperature of 25°C because the channel activation/inactivation kinetics measurements on which we base our studies were performed near room temperature; we desired to avoid unnecessary artificial scaling by a temperature factor. However, all our results were verified using values extrapolated for physiological temperatures ($Q_{10} = 2.3$), and no significant changes in behavior were observed.

Unless otherwise noted, the soma, spines, and dendrites were endowed uniformly with dendritic voltage-gated channels and given measured densities ($g_{K_{fast}} = 40 \, \text{pS/} \mu \text{m}^2$; $g_{K_{slow}} = 2.7 \, \text{pS/} \mu \text{m}^2$; $g_{Na} = 6.6 \, \text{pS/} \mu \text{m}^2$; Korngreen and Sakmann 2000; Stuart and Sakmann 1994). No gradient of $K^+$ channels was used due to the recent conflicting reports of channel distribution in layer 5 pyramidal cells (Bekkers 2000a; Korngreen and Sakmann 2000), although an addition of a gradient did not significantly change our conclusions. The voltage-gated channel models were constructed using Hodgkin-Huxley formalisms. The $Na^+$ channel model used was slightly modified from a prior study (Hoffman et al. 1997), based on experimental descriptions (Colbert and Johnston 1996; Magee and Johnston 1995), and was similar to prior backpropagation modeling studies (Mainen and Sejnowski 1996; Rapp et al. 1996; Vetter et al. 2001). Axonal densities and kinetics of $Na^+$ channels were adjusted appropriately to the physiology of spines by use of any approximation methods (Baer and Rinzel 1991; Stratford et al. 1989) since computational power of processors recently have been sufficient to handle the number of equations presented.
reproduce a characteristic somatic AP. The K conductance model incorporates both fast and slow activated channels and reflects recent experimental descriptions of the channel kinetics and densities from dendritic recordings (Bekkers 2000a,b; Korngreen and Sakmann 2000). Because our study is primarily focused on the correspondence between the model and experiments, dendritic channel parameters were kept to literature values and were not modified. For specific values used in modeling Na⁺ and K⁺ channel kinetics, please refer to Table 1. Note that axonal Na⁺ channel kinetics were modified from this scheme to reproduce spiking behavior at ɣNa = 1200 pS/μm² and ɣKfast = 360 pS/μm². Specifically the following changes were made: αm = 0.182·(v + 30)/(1 - e⁻(-(v - 30)/6)), αh = 0.08 · (v + 20)/(1 - e⁻(-(v - 20)/6)).

Clustering simulations

We compared “spine cluster,” “noncluster,” and “dendritic cluster” models. The models are constructed such that the total number of sodium channels in the “cluster” compartment is conserved; for the “spine cluster” model, the channels are distributed across the spines, for the “dendritic cluster,” channels are distributed across the apical dendrites, and for the “noncluster,” the channels are distributed across both spines and dendrites. For the spine cluster model

\[ A_g \cdot G_{sc} = (A_0 + A_g) \cdot G_{sc} \]

and for the dendritic cluster model

\[ A_d \cdot G_{dc} = (A_0 + A_d) \cdot G_{dc} \]

where \( G_{sc} \) is the spine cluster Na⁺ channel density, \( G_{dc} \) is the dendritic cluster density, \( G_{sc} \) is the spine noncluster density, and \( A_d \) and \( A_d \) are the area of the entire tree made up by the dendritic shafts and spines, respectively. The dendritic shaft density in the spine cluster model is set to the literature value (40 pS/μm²; Stuart and Sakmann 1994); the spines in the dendritic cluster model are passive. A particular cluster density in a cluster model \( G_{dc} \) can be derived from the following equation

\[ G_{dc} = \left[ \frac{(A_0 + A_d) \cdot G_{sc}}{A_d} \right] \cdot X = D \text{ or } S \]

RESULTS

Reported dendritic shaft channel densities and kinetics do not support effective backpropagation

The dendritic tree of pyramidal neurons is populated by voltage-gated ionic channels that furnish the dendrite with an excitable membrane capable of signal propagation (Johnston et al. 1996; Yuste and Tank 1996). To investigate the degree of consistency between reported membrane channel properties and backpropagation, we constructed a computational model of a mouse layer 5 neocortical pyramidal neuron (Fig. 1A). This cell type was chosen particularly due to the thorough characterization of backpropagation in this class by prior studies in vitro and in vivo (Buzsaki and Kandel 1998; Stuart and Sakmann 1994; Stuart et al. 1997a) and because of our imaging studies of its backpropagation (Holtloft et al. 2002) and anatomical analysis of their spine morphologies (Konur et al. 2001). The somatic, dendritic, and spiny compartments were endowed with active channels whose kinetics and densities were based on recent experimental descriptions (Bekkers 2000b; Korngreen and Sakmann 2000; Magee and Johnston 1995; Stuart and Sakmann 1994). The axon was endowed with moderately higher densities and modified kinetics to reproduce a characteristic AP waveform (see METHODS).

To test whether the recently reported densities of Na⁺ (40 pS/μm²) and K channels (fast -2.7 pS/μm²; slow -6.7 pS/μm²) could support effective backpropagation, we simulated an AP by somatic current injection. The AP was initiated in the axon by a current injection and the somatic response showed a standard AP waveform (Fig. 1C). However, the model was unable to support extensive backpropagation along the apical dendrite (Fig. 1A) in disagreement with experimental findings from rat layer 5 cells (Stuart and Sakmann 1994) and from our calcium imaging measurements that confirm extensive backpropagation of single AP along the apical dendrite of mouse layer 5 neurons (Holtloft et al. 2002). The waveform halfway into the dendritic tree (190 μm) was attenuated by more than 70% (Fig. 1, A–C) and failed to invade the mid- and distal dendrites; the AP pattern itself at this point became highly transformed (Fig. 1, A-C). In contrast, experimentally observed reduction of backpropagating action potentials (BAPs) in layer V pyramidal neurons have shown approximately 40% attenuation midway in the dendritic compartment (Larkum et al. 2001).

It is possible that the BAP failure in our model could be due to an underestimation of sodium current at the soma or the dendrite. Specifically, backpropagation failure could be due to insufficient sodium current injected into the soma during the

| Table 1. Na and K channel Hodgkin-Huxley model parameter values |
|------------------|------------------|------------------|
| **Na Channel**   | **K Channel (Fast)** | **K Channel (Slow)** |
| Current  \( I_{Na} = g_{Na} \cdot m^3 \cdot h \cdot (V_m - E_{Na}) \) | \( I_K = g_{K_{fast}} \cdot m^3 \cdot h \cdot (V - E_K) \) | \( I_K = g_{K_{slow}} \cdot m \cdot (0.5 \cdot h_{fast} + 0.5 \cdot h_{slow}) \cdot (V - E_K) \) |
| Gating \( \alpha_m = g_{Na} \cdot m \cdot (V - V_{half}) / (1 - e^{-((V - V_{half})/V_{offset})}) \) \( \beta_m = g_{Na} \cdot m \cdot (V - V_{half}) / (1 - e^{-((V - V_{half})/V_{offset})}) \) \( \alpha_h = g_{Na} \cdot m \cdot (V - V_{half}) / (1 - e^{-((V - V_{half})/V_{offset})}) \) | \( \alpha_f = 1/(1 + e^{-((V - E_{half})/V_{slope})}) \) \( \beta_f = 1/(1 + e^{-((V - E_{half})/V_{slope})}) \) \( \alpha_s = 1/(1 + e^{-((V - E_{half})/V_{slope})}) \) | \( \alpha_f = 1/(1 + e^{-((V - E_{half})/V_{slope})}) \) \( \beta_f = 1/(1 + e^{-((V - E_{half})/V_{slope})}) \) \( \alpha_s = 1/(1 + e^{-((V - E_{half})/V_{slope})}) \) |
| Activation \( C_{m_{max}} = 0.182 \) \( C_{m_{max}} = 0.124 \) \( C_{m_{max}} = 0.08 \) \( C_{m_{max}} = 0.0005 \) \( V_{m_{half}} = -32.5 \text{ mV} \) \( V_{m_{slope}} = 4.5 \text{ mV} \) | \( m_{max} = 0.08 \) \( m_{max} = 0.0005 \) | \( m_{max} = 0.08 \) \( m_{max} = 0.0005 \) |
| Inactivation \( C_{d_{max}} = 0.08 \) \( C_{d_{max}} = 0.0005 \) \( V_{d_{half}} = -40 \text{ mV} \) \( V_{d_{slope}} = 3 \text{ mV} \) \( V_{d_{slope}} = 5 \text{ mV} \) | \( d_{max} = 0.08 \) \( d_{max} = 0.0005 \) | \( d_{max} = 0.08 \) \( d_{max} = 0.0005 \) |
axonal AP (a “weak” somatic AP). We ruled out this possibility by first simulating a voltage-clamped 100-mV AP at the soma (with axon detached), and then immediately afterwards releasing the voltage clamp and calculating the dendritic response under current clamp. In this situation, the extent of backpropagation did not differ significantly from the original model (data not shown).

An alternative possibility to explain the backpropagation failure could be an underestimation of the maximal sodium current measured in dendritic patches. For example, the measured peak current of 7 pA could correspond to channel densities higher than 40 pS/\mu m^2 if Na⁺ channels had different kinetics and gating variables than those assumed by Stuart and Sakmann (1995). Therefore we consider it important to examine whether our model underestimated the maximal Na⁺ current measured. Using our kinetic and gating variables (see METHODS), Na⁺ currents were generated in a 1-\mu m² patch by depolarizing steps to −10 mV from a holding potential of −90 mV. Compared to experimentally measured peak currents of 7 pA (Stuart and Sakmann 1994), the sodium channel model at a density of 40 pS/\mu m² generated peak currents of approximately 24 pA/\mu m², indicating, if at all, even a possible overestimation of Na⁺ channel density. This potential overestimation does not significantly change our conclusions.

These results suggest that systematic errors of maximal current generation in our sodium channel model did not underlie the backpropagation failure.

We also investigated whether extensive dendritic branching underlaid the backpropagation failure. Interestingly, the attenuation in our model occurred well before the apical dendrite branched extensively, indicating that increased dendritic branching of the cell was not the main factor which compromised the backpropagation. To explore whether the BAP attenuation was due to a specific dendritic morphology, we also simulated backpropagation in a reconstructed rat CA1 pyramidal neuron (Pyapali et al. 1998) and still observed severe attenuation (79.6% reduction at 37.6% of dendritic length) despite the differences in branching architecture and overall length of cell. This indicated that charge flow was not impeded due to our particular dendritic morphology.

**Backpropagation sensitivity to channel densities and passive parameters**

Why do voltage channels not support efficient backpropagation in our model? We investigated this by examining the relationship between the AP attenuation and the somato-dendritic Na⁺ and K⁺ channel densities (Fig. 2). As in a previous backpropagation modeling study (Vetter et al. 2001), we used the AP height at approximately 190 \mu m from the soma as an indicator of the degree of attenuation along the apical dendrite. As expected, the Na⁺ channel density (0–200 pS/\mu m²) showed a direct sigmoidal relationship with the AP height (Fig. 2A). However, the half-maximum point of this curve lay around 75–85 pS/\mu m², about twice the value of reported physiological densities (Stuart and Sakmann 1994). At the reported density of 40 pS/\mu m², the Na⁺ current in the dendrite was insufficient to depolarize to threshold level due to the severe voltage attenuation. Instead, a channel density near 105 pS/\mu m² was needed to reproduce the experimentally observed 40% attenuation midway along the dendrite (Larkum et al. 2001).

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attenuated with higher somato-dendritic K\(^+\) channel density (0–100 pS/µm\(^2\)), although this effect was small. Interestingly even without K\(^+\) channels, severe attenuation still occurred along the apical dendrite: removing K\(^+\) channels could not increase the BAP to the same levels as when Na\(^+\) channel density was scaled to high densities (>150 pS/µm\(^2\)). After scaling the Na\(^+\) channel density severalfold to 120 pS/µm\(^2\), however, a full sigmoidal dependence on K\(^+\) channel density was revealed, indicating that the influence of these channels was only significant in models with higher Na\(^+\) channel activation. At Na\(^+\) channel densities >120 pS/µm\(^2\), the attenuation effect of increasing K\(^+\) channel density diminished due to the overwhelming activation of Na\(^+\) channels. At the measured Na\(^+\) channel densities (40 pS/µm\(^2\)), our results indicate that an overestimate of K\(^+\) channel density or conductance was not the cause of AP attenuation and support the hypothesis that the sodium channels are insufficiently activated.

We also checked that our passive parameters were not responsible for BAP failure. By modulating membrane capacitance, membrane resistance, and intracellular resistivity, we found that even extreme values could not allow effective backpropagation (Fig. 2C). Thus we consider it unlikely that the passive properties of the model caused the backpropagation failure. Taken together, these simulations thus pointed at the Na\(^+\) channel model and density as underlying the backpropagation failure along the apical dendrite.

**Backpropagation sensitivity to Na\(^+\) channel activation and inactivation kinetics**

Na\(^+\) current can be modulated by a number of neurotransmitters (Cantrell et al. 1996; Numann et al. 1991) and this can determine neuronal excitability and backpropagation (Colbert and Pan 1999). We therefore investigated the behavior of the Na\(^+\) channel model itself and its influence on backpropagation using the maximum depolarization in the apical dendrite (at 190 µm) again as an indicator (Fig. 3). Boltzmann equations were used to characterize the sodium channel currents using Hodgkin-Huxley style kinetics. Therefore only two parameters (\(V_{\text{half}}\) and Slope) were needed to describe a specific functional component of the voltage-gated channel. To simulate modulation, we varied both parameters for the activation and inactivation regimes while keeping the other components constant and analyzed their specific relationship with backpropagation efficacy.

Backpropagation showed a differential degree of sensitivity to the activation parameters (Fig. 3A). When varying \(V_{\text{half}}\) (−80 to −10 mV), attenuation in the BAP at 190 µm from the soma showed a sigmoidal dependence with the greatest sensitivity occurring in the threshold range (−35 to −55 mV). Similarly the slope parameter when varied (2–8 mV) influenced backpropagation significantly within the same region, although to a lesser degree than \(V_{\text{half}}\). The relative insensitivity to slope indicated that increasing the amount of activation per

![FIG. 3. Backpropagation sensitivity to Na\(^+\) channel kinetic parameters. The standard Na\(^+\) channel model (see METHODS) was modified by varying the \(V_{\text{half}}\) and slope parameters of a particular component described by a Boltzmann curve. The color maps plot the effect of modifying the parameters in the axis on the AP height as measured halfway along the apical dendrite (190 µm from soma). Color scale bar indicates the ratio of the height of the dendritic response compared with the height of the somatic AP; the arrow on the color scale indicates the physiologically observed ratio. Circles within the plots indicate the original experimentally measured parameters. A: backpropagation sensitivity to activation. B: backpropagation sensitivity to inactivation steady state. C: backpropagation sensitivity to inactivation entry rate \(a\). D: backpropagation sensitivity to inactivation recovery rate \(b\).](https://www.jn.org)
millivolt of depolarization was not sufficient to promote backpropagation. Furthermore, in the suprathreshold (≥ −35 mV) and subthreshold regions (< −50 mV), backpropagation was not sensitive to either slope or V<sub>half</sub> with attenuation, remaining steady within the region. As expected, extensive backpropagation was observed when V<sub>half</sub> was set in the subthreshold range due to heightened activation. It should be noted that experimental values (Huguenard et al. 1989; Magee and Johnston 1995) lie at the border of the sensitive region (Fig. 3A, circle), suggesting that moderate modulation of the V<sub>half</sub> point can produce significant changes in Na<sup>+</sup> current as found in experiment (Dascal and Lotan 1991).

Na<sup>+</sup> channel inactivation was analyzed by separate components: steady-state voltage dependence, inactivation entry rate (α), and inactivation recovery rate (β). In varying inactivation, backpropagation showed greater sensitivity to the V<sub>half</sub> parameter than the slope for all the components except for inactivation recovery rate (Fig. 3, A–C). As in activation, attenuation was most variable in the threshold range, but showed a more graded response in the suprathreshold and subthreshold regions. Interestingly the measured values lay in a region where modulation showed little influence on backpropagation, suggesting that regulation of the voltage dependence releases inactivation to a minimal extent.

Inactivation entry and recovery exhibited drastically different influences on backpropagation. While entry rate showed a sigmoidal dependence, modulation of the recovery rate did not influence attenuation of the AP as backpropagation always failed independent of parameter values (Fig. 3D). However we attribute this to the fact that this study focuses on single AP invasion; activity-dependent backpropagation is likely to be dependent on this component. Entry rate (α) showed greater sensitivity in the suprathreshold region with backpropagation approaching full invasion as V<sub>half</sub> approaches 0 mV. The slope parameter showed very little variation with attenuation varying only by a few millivolts. The original parameter values lie at the border of the threshold/suprathreshold region indicating that slight modification of the V<sub>half</sub> parameter of inactivation entry rate would promote backpropagation.

**Backpropagation sensitivity to Na<sup>+</sup> channel inactivation time constant**

Our results therefore suggested that backpropagation efficacy is highly sensitive to the inactivation entry rate (α) particularly when the half voltage parameter is modulated in the suprathreshold range (Fig. 3C). The original parameters (Table 1) lie close to the sensitive region of the entry rate modulation space, and thus, changes in this parameter are likely to exert significant influence on backpropagation extent. We therefore examined the inactivation time constant (τ<sub>h</sub>) dependence on backpropagation while modulating α and compared the curves to experimental measurements (Fig. 4).

The original model inactivation τ curve was typical and representative of prior experimental and modeling studies (Fig. 4A). The Na<sup>+</sup> channels showed preferential entry into slow inactivation in the subthreshold range (Pan and Colbert 2001) with fast inactivation occurring in the suprathreshold range (Huguenard et al. 1988; Magee and Johnston 1995), indicating that the channel model was capable of activity-dependent backpropagation. Modulating the α half activation parameter by 5–10 mV shifted the curve peak by roughly the same amount (Fig. 4A, red and blue) while increasing the peak height. Subthreshold value changes were relatively small (36–37 ms at −60 mV, +10 mV shift) compared with three- to fourfold changes in the suprathreshold region (2.2–11.3 ms at −40 mV, +10 mV shift, Fig. 4B). Since inactivation entry is slow in the subthreshold region (>20 ms), marginal increases in the subthreshold time constants does not affect the extent of backpropagation. In fact recent modeling studies which did not include slow inactivation in subthreshold regions (Mainen and Sejnowski 1996; Rapp et al. 1996) still exhibit high backpropagation efficacy. In our results, extensive dendritic propagation was supported only when the suprathreshold entry into inacti-

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**FIG. 4.** Experimental measurements of inactivation time constant and modeling of its backpropagation sensitivity. A: plots of inactivation time constant while modulating entry rate α via changing the half activation voltage (V<sub>half</sub>). B: zoom view of A in the suprathreshold range (V ≥ −40 mV). Note that a scatter plot of experimental measurements is superimposed for comparison. Data taken by Dr. C. Colbert, University of Houston, Houston, TX.
vation was prolonged. In addition we find that backpropagation is most sensitive to the inactivation time constant in the suprathreshold range due to its high variability. Thus modulation of this parameter would greatly affect backpropagation extent and dendritic membrane excitability.

We further sought to identify which parameter values best represented physiological values. Experimental steady-state inactivation in the suprathreshold range from dendritic Na\(^+\) ensembles (C. Colbert, unpublished data from CA1 pyramidal neurons) were measured and fitted by a single exponential time constant (1.6745 ms at \(-35\) mV; 0.8322 ms at \(-25\) mV; 0.5969 ms at \(-15\) mV; 0.4174 ms at \(-5\) mV; 0.3499 ms at 5 mV). Surprisingly, the results indicated that our original model values (Fig. 4B) were appropriate indicating that backpropagation should fail at approximately 30°C. Inactivation becomes very rapid in the suprathreshold range where the majority of channels will enter the inactivated state within a millisecond. These results indicate that the Na\(^+\) channel kinetic model is only weakly excitable at a density of 40 pS/\(\mu m^2\) and unable to support extensive backpropagation.

**Backpropagation sensitivity and efficiency due to highly excitable spines**

Our findings therefore suggest that dendritic shaft Na\(^+\) channels are unable to support backpropagation at the measured density of 40 pS/\(\mu m^2\). What then is the underlying support of effective backpropagation in pyramidal cells? The advent of dendritic recording techniques (Stuart et al. 1993) have allowed thorough characterization of the ion channel densities and distribution along the dendritic shaft of the proximal apical dendrite (Bekkers 2000a; Christie et al. 1996; Hoffman et al. 1997; Korngreen and Sakmann 2000; Magee and Johnston 1995). However, an unprobed area of membrane that remains are the numerous spiny structures which cover the dendritic tree and could not only provide a large membrane area but also have a different complement of channels and receptors. Since in our model spines contribute 50% of the total membrane, we proceeded to investigate the possibility of spines with excitability greater than that of the dendritic shaft, a notion previously raised in the literature before measurements of dendritic channel densities became available (Baer and Rinzel 1991; Miller et al. 1985; Rall 1988; Shepherd et al. 1985), and, in particular, the effect of excitable spines on backpropagation.

In all previous simulations we had maintained identical channel densities in spines and dendritic shafts. We now proceeded to examine the consequences of higher excitability in spines by varying spine Na\(^+\) channel density while keeping the dendritic shaft density at 40 pS/\(\mu m^2\). All other parameters were kept the same. When varying spine Na\(^+\) channel density, backpropagation efficacy showed a similar sigmoidal dependency as when varying dendritic density (Fig. 5A; compare with Fig. 2A). Surprisingly, the half-maximum point was reached at only modestly higher densities (100–120 pS/\(\mu m^2\)). These values are approximately threefold higher compared with the physiological density measured in dendritic shafts, suggesting that only a relatively small increase is needed in sodium channels of spines compared with dendrites to support nondecremental backpropagation. A fourfold higher density (160 pS/\(\mu m^2\)) of Na\(^+\) channels in spines was needed to reproduce the experimentally observed 40% attenuation. At approximately threefold dendritic shaft densities, we found that backpropagation was extensive and invaded practically all the apical tree save the distal dendrites (Fig. 6A). To reproduce full distal invasion of the dendrite, the spine channel density needed to be scaled to 200 pS/\(\mu m^2\) (Figs. 5B and 6B). With such densities, distal dendritic Na\(^+\) APs were observed in our simulations. A dendritic forward-propagating AP could be initiated only by strong dendritic current injection to depolarized levels near 0 mV (not shown); spikes were usually initiated not at the site of injection (190 \(\mu m\) from soma) but distally in smaller dendrites.

We also investigated the effect of a nonuniform distribution of Na\(^+\) channels among spines. Increasing the density of channels (700 pS/\(\mu m^2\)) along a particular distal branch produced preferential activation of branches distally due to a regenerative local dendritic spike (Fig. 6C). Therefore the local

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**FIG. 5.** Influence of spine excitability on backpropagation. A: backpropagation sensitivity to spine Na\(^+\) channel density indicated by plot of AP height ratio as measured halfway along the apical dendrite (190 \(\mu m\) from soma). Spines were endowed with higher densities of Na\(^+\) channels while the dendritic shaft density was kept at 40 pS/\(\mu m^2\). B: scatter plot of AP height ratio with spine Na\(^+\) channel density of 140 pS/\(\mu m^2\). This plot depicts the height ratio of the AP at various distances from the soma in the apical tree.
spine Na\textsuperscript{+} channel distribution, which may be related to overall synaptic strength, is important in determining the backpropagation efficacy along a particular branch.

This estimate of spine Na\textsuperscript{+} channel density might underestimate the true existing density due to several considerations. As reported above, the peak Na\textsuperscript{+} channel current probably overestimates the literature value, suggesting that the dendritic sodium channel density could be even lower than 40 pS/\textmu m\textsuperscript{2} given a more accurate match of our model kinetics. Therefore an even greater number of Na channels on spines would be needed to compensate for a decreased peak Na current value, given the same channel kinetics as in our model. In addition, if a K\textsuperscript{+} channel gradient (Bekkers 2000a) or a "leaky" apical dendrite (Stuart and Spruston 1998) exists, higher densities of spine sodium channels would be needed due to the increased distal shunt. These factors reinforce our conclusion that higher spine Na\textsuperscript{+} channel densities must exist, and indicate that our values reported here may be underestimates.

**Clustering of Na\textsuperscript{+} channels in spines facilitates backpropagation**

Although our simulations indicated that backpropagation can be supported by higher Na\textsuperscript{+} densities, we questioned whether this effect was solely due to the increased density of Na\textsuperscript{+} channels or to their local clustering in spines. To test this, we constructed three models: a "noncluster" model, a "spine cluster" model, and a "dendritic cluster" model. The noncluster model (also used in the results reported so far) was endowed with a uniform density of Na\textsuperscript{+} channels across both spine and dendritic membranes; in the cluster models these channels were placed on either the spines or the dendritic shafts. All three models were constructed such that these compartments contained exactly the same total number of Na\textsuperscript{+} channels (see METHODS). Varying the total number of channels, we examined the ratio of dendritic AP amplitudes at the midpoint along the apical dendrite between the models, thus comparing the relative backpropagation extent between the models.

Greater BAP occurred in the spine cluster model, with up to a 53% greater peak amplitude observed in comparison to both dendritic cluster and noncluster models (Fig. 7A). Peak efficiency (i.e., ratio of AP amplitude in spine cluster vs. noncluster model) was observed at spine cluster density of 128 pS/\textmu m\textsuperscript{2} (equivalent noncluster model density of 65 pS/\textmu m\textsuperscript{2}) due to the fact that depolarizations at the midpoint of the dendrite were just below the threshold range for the noncluster model (Fig. 7B). This increased activation was observed primarily in lower density ranges (0–100 pS/\textmu m\textsuperscript{2} noncluster model density) and disappeared for higher densities as Na\textsuperscript{+} channel activation saturated. In fact, backpropagation extent in the dendritic cluster model slightly surpassed that in the spine cluster model at very high densities (>100 pS/\textmu m\textsuperscript{2} noncluster model density). Differences in peak AP amplitudes between spines located midway along the dendrite and their neighboring dendritic shafts were <0.05 mV (Fig. 7C) at a cluster density of 128 pS/\textmu m\textsuperscript{2}.

We concluded that the spine head model showed much greater activation than either the dendritic cluster or noncluster model, suggesting that clustering in spines may be an energetically efficient method of sodium channel distribution.
Clustering efficiency is due to unique spine morphology

What exactly about spines allows the greater efficiency due to clustering? Since the difference between the dendritic and spine head sites primarily is one of electrical environment, we proceeded to investigate whether or not spine head impedance or neck resistance affected backpropagation efficacy via changes in spine morphology.

To change spine head impedance, we varied head diameter while keeping the total Na⁺ channel number constant on the spine head. In this way, we were able to isolate the effects of changing spine input resistance without increasing Na⁺ conductance. When varying head diameter (0.2–4.0 μm), backpropagation efficacy dropped dramatically as the spine head diameter increased (Fig. 8A). The lower input resistances of larger spine heads prevented sufficient activation of Na⁺ current leading to backpropagation failure (9%–0% BAP height ratio, 2.0–4.0 μm). In contrast, changes in the higher input resistance of smaller head diameters had a much larger effect on backpropagation attenuation (73%–9% AP height ratio, 0.2–2.0 μm).

We also found that backpropagation was sensitive to changes in neck resistance globally. Backpropagation efficacy decreased significantly as a function of increasing neck length (0–10 μm, 0–254 MΩ; Fig. 8B). Backpropagation sensitivity to neck resistance was relatively more important in the 50–100 MΩ range. Overall, increasing neck lengths ≥10 μm reduced dendritic AP height from approximately 48% to 19% of the somatic amplitude, indicating that the spine Na⁺ channel activation is possible by varying neck lengths. To address whether this result was due to increasing neck membrane area, we also simulated various neck lengths while changing neck diameter such that total membrane area remained constant; varying neck length in the same range (0–10 μm), we found

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that dendritic AP height decreased dramatically (57%–30%) as resistance increased (results not shown). Thus the decreases in backpropagation efficacy were not due to the increased area of the neck membrane or increases in total cell capacitance.

In the prior section, we reported that clustering channels in spines allowed increased backpropagation efficacy (Fig. 7). After lowering spine head impedance and/or increasing neck resistance, we were able to dissipate this increased activation returning the cell to backpropagation failure levels, indicating that the efficiency of clustering is due primarily to the spine’s electrical properties that are determined by its morphology (Fig. 8). Since increasing the spine head diameter globally by 50% produces a marked attenuation (approximately 30% decrease in height ratio, Fig. 8A), the high-input impedance of the spine head must offer a more conducive activation environment than the relatively low impedance of the dendritic shaft; moreover, low neck resistances allow almost full charge transfer from the dendrite to the spine head (Fig. 7C). Our results indicate that changes in spine geometry could significantly alter backpropagation.

DISCUSSION

Backpropagation has been shown to be influenced by dendritic morphology (Stuart et al. 1997b), passive properties (Stuart and Spruston 1998), synaptic activity (Rapp et al. 1996), and ion channel density and spatial distribution (Hoffman et al. 1997). Prior modeling studies (Mainen and Sejnowski 1996; Rapp et al. 1996; Vetter et al. 2001) used simulations based on cable theory combined with nonlinear mechanisms; however, these studies have not explored backpropagation dependence on modulations in Na⁺ channel kinetics. Because backpropagation can be modified via channel modulation by neurotransmitters (Hoffman and Johnston 1999), it is important to explore what channel kinetics most influence dendritic signal propagation. In this study we investigate the backpropagation behavior of a particular model scheme based on measurements of dendritic channel densities (Bekkers 2000b; Korngreen and Sakmann 2000; Magee and Johnston 1995), identify the most backpropagation-sensitive channel kinetics, and explore the role of excitatory spines in dendritic signal propagation.

Our results highlight the importance of spines in backpropagation as primary sites of Na⁺ channel modulation. As recent studies have demonstrated the control of backpropagation in pyramidal neurons by modulation (Hoffman and Johnston 1999), here we show that the key sensitive component of single BAP attenuation is the Na⁺ channel inactivation time constant in the suprathreshold range. Furthermore, experimental measurements of this parameter indicate that measured dendritic shaft densities surprisingly cannot support extensive backpropagation alone. We demonstrate that excitatory spines can play a necessary and efficient role in supporting effective backpropagation, and that it is the unique morphology of spines which allows greater activation efficiency. In this work, we make the assumption that spine Na⁺ channels are identical to those present in the dendritic shaft. As an alternative scenario, without recurring to assume higher Na⁺ channel densities, it is also possible that spines have Na⁺ channels that differ importantly in their kinetics, particularly in the suprathreshold inactivation entry or activation curve, and this could enable backpropagation. Although the Na⁺ channel density in apical dendrites has been measured to be constant (Magee and Johnston 1995; Stuart and Sakmann 1994), we propose that the degree of global and local postsynaptic clustering of Na⁺ channels in spines plays an important role in backpropagation behavior.

Suprathreshold inactivation entry regulates backpropagation

A surprising result of our study is that the measured densities of voltage-gated channels on the dendritic shaft are unable to support effective backpropagation due to fast inactivation in the suprathreshold range. This contrasts with prior modeling studies that have demonstrated full backpropagation supported by physiological densities (Mainen and Sejnowski 1996; Rapp et al. 1996; Vetter et al. 2001). Our Na⁺ channel model is similar to those used in these studies, but differs importantly in the suprathreshold values of inactivation time constant. We used experimental measurements in this range to support our premise that fast suprathreshold inactivation can occur in pyramidal cells, although this may vary in individual cells.

The current state of inactivation appears to be an important indicator of the degree to which backpropagation can be supported. Recently, several studies have shown that trains of BAPs attenuate in an activity-dependent manner due to the slow inactivation of Na⁺ channels (Callaway and Ross 1995; Colbert et al. 1997; Mickus et al. 1999; Spruston et al. 1995). Specifically, the state of slow inactivation shapes the degree of attenuation of the signal as well as dendritic excitability. Our results complement these findings by demonstrating that inactivation entry is the key parameter in determining dendritic responses to APs. While subthreshold entry may allow synaptic activity dependent modulation of backpropagation (Pan and Colbert 2001), we suggest that suprathreshold entry may similarly encode the output activity of the cell.

Existence of Na⁺ channels in dendritic spines and influence on dendritic signaling

We propose that an efficient solution to this backpropagation failure is the existence of excitable spines with Na⁺ channel densities three- to fivefold greater than that of the dendritic shaft. Although no direct evidence has shown Na⁺ channels exist in spine membrane, we believe that this is a reasonable prediction based on the robustness of the simulations to varying parameter space. Immunocytochemical studies have suggested the existence of Na⁺ channels at spine cytoplasm (Calo et al. 2000) while specific subtypes of voltage-gated calcium channels have been found to be present at spines membranes (Sabatini and Svoboda 2000; Yuste and Denk 1995). Theoretical studies have suggested that voltage-gated channel density at spines can be related to synaptic weight (Koch and Poggio 1983; Segev and Rall 1998; Wu and Baer 1998) and could support dendritic signal propagation (Baer and Rinzel 1991; Shepherd et al. 1985). Our findings extend this work suggesting that the spine head membrane conductances may play a major role in supporting dendritic signals not only due to its membrane contribution, but also to its major contribution of highly active Na⁺ channels.

The overall clustering of Na⁺ channels in spines promotes current propagation in the apical tree (Fig. 7) due to the unique high-impedance environment of the spine head (Fig. 8).
sides enabling backpropagation, a higher density of spine Na\(^+\) channels presents additional advantages. Clustering is an efficient strategy as activation is maximized using a smaller number of channels. This strategy, evident in the axon membrane, may have independently evolved in dendrites. Also, the increased activation due to postsynaptic clustering may help mitigate the shunting effect of synaptic activity (Rapp et al. 1996). Moreover, clustering can facilitate the amplification of excitatory postsynaptic potentials (EPSPs), thereby facilitating a location-independent somatic response (Magee and Cook 2000). Indeed excitatory spines, by enabling clustering of excitable membrane conductances, may represent a backbone on which dendritic signal amplification occurs.

Although clustering of spine Na\(^+\) channels provides an efficient solution to the backpropagation failure, we do not discount the equally likely possibility that spine Na\(^+\) channels differ in subunit composition than dendritic channels. Recent work has shown that specific subtypes of voltage-gated Ca\(^{2+}\) channels (R-type) are functionally important only in spine membranes, while other subtypes (L-, N-, and P/Q-type) found in dendrites are not (Sabatini and Svoboda 2000). Similarly a different subtype of Na\(^+\) channel that facilitates current generation may be targeted to the spine head. Future experiments will be needed to determine which scenario (clustering or subtype) exists.

Finally a particularly interesting result of our simulations is that global spine morphology significantly influences backpropagation efficacy. Spine neck resistances are estimated to be in the 4–50 M\(\Omega\) range (Svoboda et al. 1996), indicating that spines are not able to compartmentalize voltage effectively from the dendritic shaft. However, our study has shown that despite a submillivolt differential between spine and dendrite, changes in neck length and/or head diameter at an extensive level can modulate dendritic signaling behavior such as backpropagation. Thus we suggest that the impact of actin-based changes in geometry of spines (Matus 2000) may not be only restricted to chemical regulation, but also to the electrical properties of spines.

**Consequences of different spine densities in neurons**

Our findings demonstrate that the function of dendrites could be more sensitive to changes in spine density than previously thought. Given that spine density changes occur during development (Gould et al. 1990; Harris et al. 1992) and could be related to synaptic plasticity (Engert and Bonhoeffer 1999; Maletic-Savatic et al. 1999), backpropagation should be particularly sensitive to these processes. Assuming similar channel kinetics and effective backpropagation across neuronal types, spine density should have an inverse relationship with shaft Na\(^+\) channel density. The fact that backpropagation failure also occurred in a reconstructed CA1 pyramidal neuron suggests that these relationships may extend to pyramidal cell types in general.

As spine density may be an indicator of Na\(^+\) channel clustering, in less spiny cell types such as nigro-striatal neurons and cortical interneurons, effective dendritic signal propagation should be supported by dendritic Na\(^+\) channel densities several times higher than that in pyramidal dendritic shafts. Indeed, the peak Na\(^+\) conductance density of alveus-oriens interneurons, which are moderately spiny (Sik et al. 1995), has recently been shown to be approximately threefold greater than that of cortical pyramidal neurons (Martina et al. 2000). In addition to backpropagation, spine density, as an indicator of channel clustering, could affect other modes of dendritic signaling such as EPSPs, inhibitory postsynaptic potentials (IPSPs), and local spiking. Thus spiny neurons such as cortical pyramids and Purkinje cells, which are also primary output cells, could share similar methods in dendritic computation. Although we do not discount the major role of dendritic morphology and channel densities in controlling dendritic signals, spinyness could serve as an important indication of the input/output processing strategy of a neuron.

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The simulation code is available from the authors by request.

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