Dendritic Resonance in Rat Neocortical Pyramidal Cells

DANIEL ULRICH
Institute of Physiology, University of Bern, CH-3012 Bern, Switzerland

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Ulrich, Daniel. Dendritic resonance in rat neocortical pyramidal cells. J Neurophysiol 87: 2753–2759, 2002; 10.1152/jn.01000.2001. Dendritic integration of synaptic signals is likely to be an important process by which nerve cells encode synaptic input into spike output. However, the response properties of dendrites to time-varying inputs are largely unknown. Here, I determine the transfer impedance of the apical dendrite in layer V pyramidal cells by dual whole cell patch-clamp recordings in slices of rat somatosensory cortex. Sinusoidal current waveforms of linearly changing frequencies (0.1–25 Hz) were alternately injected into the soma or apical dendrite and the resulting voltage oscillations recorded by the second electrode. Dendrosomatic and somatodendritic transfer impedances were calculated by Fourier analysis. At near physiological temperatures (T ~35°C), the transfer impedance had a maximal magnitude at low frequencies (f_{res} ~6 Hz). In addition, voltage led current up to ~3 Hz, followed by a current lead over voltage at higher frequencies. Thus the transfer impedance of the apical dendrite is characterized by a low-frequency resonance. The frequency of the resonance was voltage dependent, and its strength increased with dendritic distance. The resonance was completely abolished by the I_{h} channel blocker ZD 7288. Dendrosomatic and somatodendritic transfer properties of the apical dendrite were independent of direction or amplitude of the input current, and the responses of individual versus distributed inputs were additive, thus implying linearity. For just threshold current injections, action potentials were generated preferentially at the resonating frequency. I conclude that due to the interplay of a sag current (I_{h}) with the membrane capacitance, layer V pyramids can act as linear band-pass filters with a frequency preference in the theta frequency band.

INTRODUCTION

Pyramidal cells are the main excitatory elements of neocortex. They are contacted by many other excitatory and inhibitory neurons via synapses, which are distributed over their somatodendritic membrane (DeFelipe and Fariñas 1992). The ultimate output of pyramidal cells consists of action potentials, which are initiated mainly at the initial segment of the axon as trains of individual spikes or spike bursts (Connors and Gutnick 1990; Stuart and Sakmann 1994). Knowledge of the precise rules by which synaptic inputs are converted into spike output is of key relevance for our understanding of neural function. Dendrites have long been thought to play an important role in processing synaptic signals, because their elongated cable-like structures allow for nonlinear interactions (Rall 1964), which may be computationally relevant (Mel 1994). The apical dendrite of pyramidal cells has been intensively investigated and serves as a paradigm for dendritic integration.

A variety of active membrane conductances have been found in dendritic recordings (reviewed in Johnston et al. 1996), which allow for retrograde propagation of action potentials as well as for boosting or attenuation of individual synaptic signals (reviewed in Häsuer et al. 2000; Magee 2000). While individual synaptic signals have very fast frequency components, neurons in vivo also encounter slower input fluctuations due to the time-varying synaptic background activity in the surrounding neural network (e.g., Steriade et al. 2001). Because it is largely unknown how dendrites respond to different temporal components of their synaptic inputs, I determine in this study the transfer impedance of the apical dendrite in layer V pyramidal cells over a physiologically relevant frequency range.

METHODS

Tissue preparation and recordings

Wistar rats of either sex (postnatal day 19–22) were killed by decapitation. Individual cerebral hemispheres were glued on a stage tilted forward by 15°. Parasaggital slices of 300-µm thickness were cut on a Microtome (Leica VT 1000 S, Nussloch, Germany) and incubated at 35°C. Slices were transferred to a recording chamber and superfused at 4 ml/min (T = 35 ± 1°C) with standard artificial cerebrospinal fluid containing (in mM) 125 NaCl, 1.25 NaH_{2}PO_{4}, 25 NaHCO_{3}, 2.5 KCl, 1 MgCl_{2}, 2 CaCl_{2}, and 10 glucose, pH 7.5 adjusted with 5% CO_{2}-95% O_{2}. Patch pipettes were filled with a solution containing (in mM) 125 K-Gluconate, 5 NaCl, 1 MgCl_{2}, 1 CaCl_{2}, 10 HEPES, and 11 EGTA. Electrode resistance and capacitance were minimized by electronic compensation. Membrane voltage was recorded in Bridge mode with Axoclamp-2B amplifiers (Axon Instruments, Foster City, CA). Voltage and current traces were low-pass filtered at 1 kHz and digitized at 2 kHz (12 bits) with a Labmaster LM-12 A/D converter (Scientific Solutions, Solon, OH). A liquid junction potential of approximately −10 mV was left uncorrected.

Data analysis

Impedance magnitude (|Z|) and phase shift (ϕ) were calculated by Fourier transformation of digitally averaged (n = 10) current and voltage traces. Data were low-pass filtered at 1 kHz. Filtered voltage and current were normalized to their mean value.

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voltage traces (Matlab, The Mathworks, Natick, MA). The complex impedance is \(Z(f) = \frac{V(f)}{I(f)}\) with a real and imaginary part: \(Z = \text{Re}(Z) + i \cdot \text{Im}(Z)\), and \(f\) is frequency (in Hz). \(|Z|\) and \(\phi\) were obtained as \(|Z| = \sqrt{\text{Re}(Z)^2 + \text{Im}(Z)^2}\) and \(\tan^{-1} \phi = \frac{\text{Im}(Z)}{\text{Re}(Z)}\), respectively. To avoid boundary effects, which may have been introduced by the finite sampling window, only frequencies between 1 and 20 Hz were considered for analysis. The strength of the resonance is \(Q = \frac{|Z_{\text{res}}|}{|Z_{\text{min}}|}\) (Koch 1984) (cf. Fig. 2), where \(|Z_{\text{res}}|\) and \(|Z_{\text{min}}|\) are the impedance magnitude at the resonance or lowest frequency, respectively.

RESULTS

To investigate the transfer impedance of the apical dendrite in layer V pyramidal neurons, dual whole cell patch-clamp recordings were obtained simultaneously from the soma and in layer V pyramidal neurons, dual whole cell patch-clamp electrodes, respectively. To avoid boundary effects, which may have been introduced by the finite sampling window, only frequencies between 1 and 20 Hz were considered for analysis. The strength of the resonance is \(Q = \frac{|Z_{\text{res}}|}{|Z_{\text{min}}|}\) (Koch 1984) (cf. Fig. 2), where \(|Z_{\text{res}}|\) and \(|Z_{\text{min}}|\) are the impedance magnitude at the resonance or lowest frequency, respectively.

FIG. 2. Dendritic resonance. A: transfer impedance magnitude. B: phase shift for dendrosomatic (straight line) and somatodendritic (dashed line) voltage transfer. Note the close superposition of the 2 traces: A: the impedance magnitude has a maximum at \(-6\) Hz (\(Z_{\text{max}}\); arrow) and falls off for de- and increasing frequencies. B: voltage leads current up to \(-3\) Hz (\(\Delta \Phi_0\); arrow) followed by current leading voltage for higher frequencies.

Transfer impedance of the apical dendrite

In the experiment of Fig. 1A a sinusoidal current waveform, which changed its frequency linearly between 0.1 and 25 Hz (chirp), was injected via the dendritic pipette. A second electrode measured the voltage response at the soma. The amplitude of the somatic voltage oscillations initially increased and peaked after around 400 ms (arrow), followed by a steady decline at higher frequencies. The reverse experiment in the same cell is shown in Fig. 1B. Here, the chirp current was injected via the somatic pipette and the voltage response recorded by the dendritic patch electrode. Again, the voltage amplitude at the dendrite is maximal after \(-400\) ms (arrow). Somatodendritic and dendrosomatic transfer impedances were calculated after Fourier transformation of digitally averaged current and voltage traces. Figure 2 (A and B) shows transfer impedance magnitude (A) and phase shift (B) for dendrosomatic (continuous line) and somatodendritic (dashed line) current flow (cf. Fig. 1). Both traces are well superimposed. The impedance magnitude has a maximum (\(Z_{\text{max}}\)) of \(-30\) M\(\Omega\) at a frequency (\(f_{\text{res}}\)) of \(-6\) Hz (Fig. 2A, arrow). Analysis of phase shift indicates that voltage leads current up to a frequency of \(-3\) Hz (\(\Delta \Phi_0\); Fig. 2B, arrow), followed by a voltage lag. At higher frequencies the voltage lag asymptotically approaches \(\pi/2\) (i.e., a quarter-cycle). Similar results were obtained in all other cells with \((f_{\text{res}}) = 5.5 \pm 1.0\) Hz and \((\Delta \Phi_0) = 2.3 \pm 0.9\) Hz (mean \pm SD; \(n = 16\)).

The membrane potential dependence of the transfer imped-
 ance was examined by simultaneously shifting the membrane voltage at both electrodes via DC current injections. Figure 3A shows the somatic voltage response to dendritic chirp inputs at three different membrane potentials ($V = -90, -80,$ and $-70$ mV). Transfer impedance magnitude ($B$) and phase shift ($C$) are shown for the three recordings in A. Note the successive decrease of impedance magnitude with hyperpolarization accompanied by a shift of $f_{res}$ to higher frequencies. Figure 4A shows the voltage dependency of $f_{res}$ in four different cells. For membrane voltages below threshold, there was an approximately linear relationship between $f_{res}$ and the membrane voltage as indicated by the straight line, which was fitted to the data points by linear regression (Pearson’s $r = -0.6, P < 0.05$). On average, the resonance frequency increased by 0.6 Hz/10 mV of hyperpolarization.

Next, the possible influence of electrode position on parameters of dendritic resonance was assessed. Figure 4B shows a scatter plot of dendrosomatic $Z_{max}$ and dendritic electrode location. As expected for a linear cable, the transfer impedance decreases with increasing distance from the soma. This is underlined by the straight line, which was fitted to the data points by linear regression (Pearson’s $r = -0.57, P < 0.05$).

In Fig. 4C the resonance frequency ($f_{res}$) is plotted against dendritic distance. Here, no correlation was found between the two parameters, suggesting site independence of the resonance frequency. Figure 4D shows a plot of resonance strength ($Q$) against interelectrode distance. Linear regression reveals a significant increase of $Q$ with somatofugal distance (Pearson’s $r = 0.62, P < 0.05$). An increase of $Q$ with distance is predicted for linear cables (Koch 1984) and is likely enhanced by an inhomogeneous distribution of $I_h$ (see following text and Discussion) (Berger et al. 2001; Williams and Stuart 2000). Linear models were fitted to the data in Fig. 4, B and D, to underline a significant correlation. However, fits from more accurate cable models did not converge, most likely due to the limited data set and the well-known cell-to-cell variability of electrical parameters.

I next aimed to characterize the pharmacological basis of the dendritic transfer impedance resonance. Neurons with passive membrane properties are characterized by low-pass filter behavior, i.e., the impedance decreases steadily with increasing frequency due to the capacitive properties of the lipid bilayer. For resonance to occur, low frequencies need additionally to be attenuated. This likely involves mechanisms other than simple passive cable properties. The subthreshold electrical properties of layer V pyramidal cells are considerably influenced by a hyperpolarization-activated cation current ($I_h$) (Stafstrom et al. 1984), which is also present in the apical dendrite (Berger et al. 2001; Williams and Stuart 2000). Figure 5A shows the somatic voltage response to dendritic chirp current injections under control conditions and in presence of $100 \mu M$ ZD 7288 (4-{N-ethyl-N-phenylamino}-1,2-dimethyl-6-{methylamino} pyrimidinium chloride), an irreversible h-channel blocker (Harris and Constanti 1995). Figure 5, B and C, shows transfer impedance magnitude ($B$) and phase shift ($C$) in control (solid line) and after ZD 7288 application (dashed line). Under control conditions, the transfer impedance shows a typical resonance behavior with a maximal impedance magnitude and voltage phase lead at low frequencies. After blockade of $I_h$ with ZD 7288, the transfer impedance now follows low-pass behavior, i.e., the impedance magnitude decreases monotonically with frequency, and the phase shift asymptotically approaches a quarter cycle. Equivalent results were obtained in three cells. Together, these results show that the resonance of the dendritic transfer impedance is mediated by $I_h$.

**Sub- and suprathreshold responses**

Despite their complex geometry and variety of ion channels present, dendrites were often found to behave linearly under subthreshold conditions (Berger et al. 2001; Cash and Yuste 1998, 1999; Ulrich and Stricker 2000). To test for linearity of the dendritic transfer function, amplitude and direction of the chirp input current were systematically varied. Figure 6A shows the somatic voltage response to two different dendritic chirp current injections. The chirp was either incrementally or decelerating. Figure 6B and C, depicts the dendrosomatic transfer impedance magnitude ($B$) and phase shift ($C$) for an accelerating (solid line) and decelerating (dashed line) dendritic chirp input. Both graphs...
show close superposition, indicating independence of the transfer impedance from the sign of the chirp. In other experiments \((n = 8)\), the amplitude of the chirp input was systematically varied. Figure 7A shows somatic voltage recordings to dendritic chirp inputs of two different amplitudes. Transfer impedance magnitude \((B)\) and phase shift \((C)\) are closely superimposed, indicating homogeneity of the dendritic transfer impedance. Figure 8A illustrates the somatic voltage response for somatic (dashed lines) or dendritic (solid lines) chirp current injections, together with the somatic response to a simultaneous somatodendritic input (dashed-dotted line). Figure 8, B and C, depicts the impedance magnitude \((B)\) and phase shift \((C)\) for somatic (dashed line), dendritic (solid line), and combined somatic-dendritic input (dashed-dotted line), together with the algebraically summed impedance of individual somatic and dendritic inputs (dotted line). The close match of the algebraically with the experimentally summed input indicates superposition \((n = 3)\). Homogeneity and superposition are key properties of linear systems.

To investigate the influence of the dendritic resonance on the generation of action potentials, sub- and suprathreshold chirp currents were injected into the dendrite and the somatic voltage responses measured. Figure 9A shows an example of a subthreshold dendritic chirp input, which led to an oscillatory somatic voltage response with the typical fusiform envelope. Successfully, the amplitude of the chirp input was slightly enhanced. Now, action potentials were detectable at the somatic electrode. Note that the time points of the maximal subthreshold voltage deflections and occurrence of spikes are coincident (arrow). Figure 9B shows a similar experiment in presence of ZD 7288. A subthreshold dendritic chirp input led to a monotonically decreasing voltage oscillation at the soma, in agreement with the low-pass filter properties of a passive dendrite. Successfully, the amplitude of the chirp input was increased, and spikes were now emitted at the peak of the lowest frequency oscillation (arrow). These results imply that under physiological conditions, pyramidal cells can behave as band-pass filters with preferential spike generation at the intrinsic resonating frequency.

**DISCUSSION**

In the present study I show that the proximal apical dendrite of rat layer V pyramidal cells behaves linearly around resting potential and is endowed with a resonance, which favors input in the theta frequency band.

**Characteristics of dendritic resonance**

The dendritic resonance was abolished by the irreversible H-channel blocker ZD 7288 and thus depends on a hyperpolarization-activated cation current \(I_h\). Indeed, \(I_h\) was found in recordings from the apical dendrite with a density that increased with more distal locations (Berger et al. 2001; Williams and Stuart 2000). Blockade of \(I_h\) was also shown to enhance the flow of “tonic” excitation from the apical dendrite to the soma (Schwindt and Crill 1997). Our data suggest an additional role for \(I_h\) as a high pass filter of dendritic input, which together with the low-pass behavior of the passive membrane leads to dendritic resonance. Indeed, a resonance frequency of \(\sim 6\) Hz is about halfway between the activation time constant of \(I_h\) \((\sim 30–100\) ms) (Berger et al. 2001; Williams and Stuart 2000) and the passive membrane time constant in these cells \((\sim 14\) ms; data not shown). However, it remains to be investigated to what degree this resonance frequency can be shifted by modulation of the membrane leak conductance or \(I_h\) (Destexhe and Paré 1999; Pape 1996).

**FIG. 4.** Parameters influencing dendritic resonance. A: the resonance frequency increases linearly with hyperpolarization. Data points from 4 different cells are fitted by a straight line (Pearson’s \(r = -0.6, P < 0.05\)). Experiments are symbol coded (■ was obtained in 1 μM TTX), B: the transfer impedance maximum \((Z_{\text{res}})\) decreases with dendritic distance. The straight line was fitted by linear regression (Pearson’s \(r = -0.57, P < 0.05\)), C: the resonance frequency \((f_{\text{res}})\) is plotted against interelectrode distance. No clear correlation between these parameters could be found. D: the strength of the resonance \((Q)\) is plotted vs. distance. The straight line was fitted by linear regression analysis (Pearson’s \(r = 0.62, P < 0.05\)) and shows an enhancement of \(Q\) with increasing somatofugal distance.
The pharmacological profile of the dendritic transfer impedance resonance in this study is compatible with a 1- to 3-Hz resonance described previously at the soma of pyramidal cells (Hutcheon et al. 1996). The lower frequency of this somatic point impedance resonance and the apparent absence of a dendritic resonance in a previous study (Ulrich and Stricker 2000) are likely to be attributed to differences in recording temperature (room temperature vs. 35°C). I measured a $Q_{10}$ of $\sim 4$ for the temperature dependence of the resonance frequency in these cells ($Q_{10} = 3.8 \pm 1.6, n = 3$, data not shown), which is compatible with the results of Hutcheon et al. (1996). Likewise, a somatic point impedance resonance of $\sim 6$ Hz has been found in hippocampal pyramidal cells at a comparable elevated recording temperature (Leung and Yu 1998). The hyperpolarization induced decrease of $Z_{\text{max}}$ and shift of $f_{\text{res}}$ to higher frequencies is in agreement with previous findings in this cell type at the soma (Hutcheon et al. 1996), and is in line with the voltage dependency of $I_h$ kinetics (Berger et al. 2001; Williams and Stuart 2000). While $I_h$ is responsible for resonance in principal cells of cortex and thalamus, in other brain regions different ionic mechanisms can cause resonance phenomena (Hutcheon and Yarom 2000; Llinás 1988).

**Resonance, dendritic integration, and physiological rhythms**

The subthreshold voltage transfer along the dendrite at rest was independent of the time course or amplitude of the chirp input. In addition, the voltage response to a distributed current input was equal to the sum of the individual inputs. These are essential features of linear systems. This result is surprising in view of the plethora of dendritic conductances in pyramidal cell dendrites (cf. Johnston et al. 1996), but in agreement with previous studies on signal propagation along the apical dendrite (Berger et al. 2001; Cash and Yuste 1998, 1999; Ulrich and Stricker 2000). Theoretically, active membrane processes are not incompatible with linearity. Many conductances behave linearly over a certain voltage range, as do dendritic cables, which have those incorporated (Koch 1984). The distance dependencies of $Z_{\text{max}}$ and $Q$ are

**FIG. 5.** A sag current underlies dendritic resonance. A: somatic voltage oscillations induced by dendritic chirp current injections are shown under control conditions and after bath application of the h-channel blocker ZD 7288 (100 μM). Note the transition of the voltage response from band-pass (top, control) to low-pass (bottom, ZD 7288) behavior. B and C: transfer impedance magnitude and phase shift are shown for the experiment in A. B: note that ZD 7288 transforms the convex frequency dependence of the transfer impedance magnitude under control conditions (solid line) into a monotonically decreasing relationship (dashed line). C: the positive phase shift for low frequencies under control conditions (solid line) is abolished by ZD 7288 (dashed line).

**FIG. 6.** Waveform invariance of dendritic transfer impedance. A: accelerating or decelerating chirp current waveforms were injected into the apical dendrite and the voltage responses recovered by a somatic electrode. B and C: impedance magnitude (B) and phase shift (C) for accelerating (solid lines) and decelerating (dashed lines) chirp inputs are superimposed. The close match of both graphs indicates that the electrical behavior of the dendrite is independent of the sign of the chirp.
compatible with linear cable theory (Koch 1984) and may be pronounced due to the increased density of $I_h$ at more distal dendritic segments (Berger et al. 2001; Williams and Stuart 2000). From these results it can be predicted that although the impact of distal input is weaker (smaller $Z_{max}$), the resonance frequency will be better discriminated (increased $Q$). Two limitations may restrict the generalization of this study to the overall input-output behavior of pyramidal cells. 1) Simple current injections were used to perturb the cells. This will leave nonlinearities associated with synaptic conductances undetected (Rall 1967). 2) Recordings were limited to relatively proximal segments of the apical dendrite. It has been shown that the distal apical dendrite has peculiar electrical properties, which may lead to a more complex input-output relationship for inputs to the apical tuft (e.g., Larkum et al. 2001). Also, it remains to be shown whether and under what conditions linearity applies to basal and oblique dendrites (cf. Oakley et al. 2001).

The increased impedance at the resonating frequency should favor the propensity of the neuron to generate action potentials at that frequency (Hutcheon and Yarom 2000). This has been confirmed in the present study for just threshold current amplitudes (Fig. 9). Preferred spiking of pyramidal cells around 6 Hz has been previously described in slices of rat somatosensory cortex (Silva et al. 1991).

Given the strong temperature dependence of the resonance, such rhythmic spiking is more likely to occur around 9 Hz in vivo (extrapolated from 35°C to a body temperature of 38°C with a $Q_{10} \sim 4$, see above), i.e., in the alpha band. In a recent study, associations of primary sensory input with signals from higher brain areas have been shown to occur through synchronizations in the alpha band (von Stein et al. 2000). Such associations could be favored by the resonance described in this study, particularly because the strength of the resonance increases with somatofugal distance. Rhythmic discharges in sensory cortex within the alpha frequency band were also found in rats before and during exploratory behavior (Nicolelis et al. 1995). Our data show that the frequency tuning of pyramidal cells, which results from their intrinsic input-output properties, is compatible with the behavior of the cortical network during sensory processing.

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FIG. 7. Homogeneity of dendritic transfer impedance. Two chirp current waveforms with nearly duplicating amplitude ratio were successively injected into the apical dendrite and the voltages oscillations recorded at the soma. B and C: transfer impedance magnitude (B) and phase shift (C) are plotted for both current inputs. Note the close superposition of the 2 traces, thus implying homogeneity.

FIG. 8. Superposition of dendritic transfer impedance. A. left: a chirp was injected successively through the somatic or dendritic pipette, followed by a simultaneous current injection via both pipettes. The voltage responses at the soma are shown for the 3 input configurations (right). The middle trace (solid line) shows the somatic voltage response to the dendritic chirp input. A similar response of slightly higher amplitude results from the somatic chirp injection (bottom dashed trace). The combined dendritic and somatic input leads to the somatic voltage response shown in the top right graph (dashed-dotted line). B and C: impedance magnitude and phase shift for the 3 experiments in A.

Dendrosomatic transfer impedance (solid line) and somatic point impedance (dashed line) are shown together with their algebraic sum (dotted line). Superimposed is the transfer impedance for concomitant somatic and dendritic input (dashed-dotted line). Note the close superposition of the impedance for the summed current input with the algebraically summed impedances of either input alone.

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FIG. 9. Band-pass spiking behavior. A: 2 chirp current waveforms of increasing amplitude were successively injected into the apical dendrite of a layer V pyramidal cell. A 2nd pipette measured the voltage at the soma. The smaller voltage response remained subthreshold and had a maximum amplitude after ~400 ms. The larger current input lead to the emission of action potentials. Note the coincidence of preferred spiking with the maximal subthreshold voltage deflections. Action potentials are truncated due to averaging. B: A similar experiment is shown in presence of the h-channel blocker ZD 7288 (100 μM). A subthreshold dendritic chirp input generates monotonically decreasing voltage oscillations at the soma. Another larger dendritic chirp input initiated action potentials at the 1st depolarizing voltage deflection (arrow). Again, spikes are truncated due to averaging.

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