

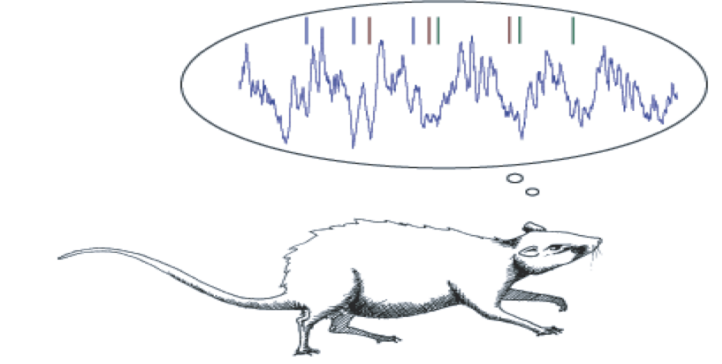
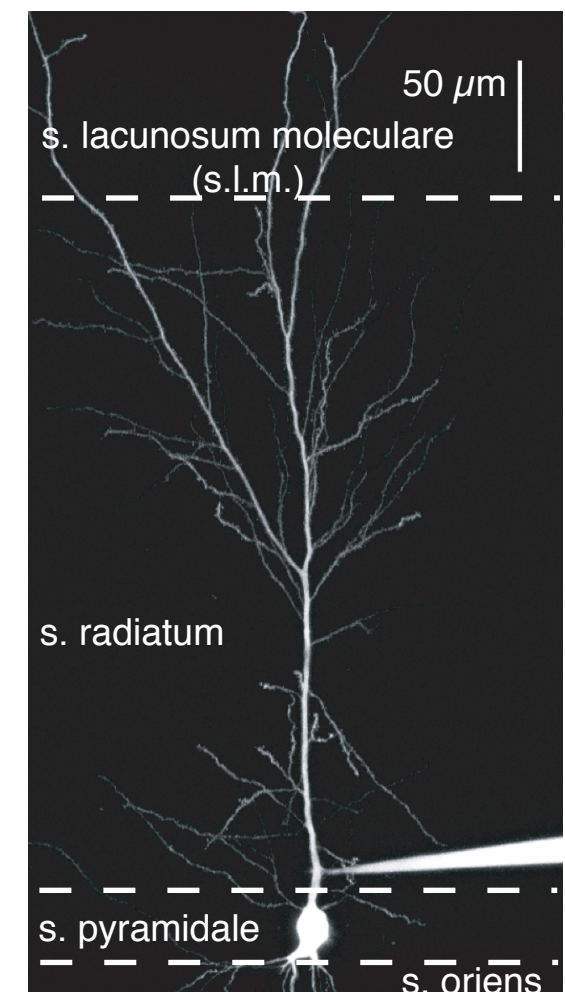
Abstract

A typical CA1 neuron in the rodent hippocampus receives about 20,000 (mostly) excitatory synapses in the s. radiatum region (1), which spans about 350 μm from the soma to the start of s.l.m., receiving primarily inputs from the CA3/CA2 region. An obvious challenge for the CA1 neuron is to integrate these inputs across this expansive region simultaneously with no differences in timing based on their spatial distribution. So, how does a CA1 neuron reach across hundreds of micrometers without compromising the temporal resolution of the incoming inputs?

To answer this question, we first considered the spatiotemporal nature of inputs that the CA1 neuron experiences during the active state of the hippocampal network. Studies have shown that whenever the rat is involved in an active voluntary activity, be it foraging for food or processing of sensory stimuli like odor or touch, the hippocampus enters a network state called 'Theta rhythm' which is characterized by slow oscillations in the 4-10 Hz frequency range (2). Furthermore, intracellular recordings during this 'online' state of the network, show that the CA1 neuron receives periodic excitation and inhibition resulting in theta frequency (4-10 Hz) membrane potential oscillations in the dendrites as well as the soma (3).

Here we show that the CA1 neuron exploits these slow rhythmic inputs such that inputs across the apical dendrite reach the integration zone near the soma simultaneously irrespective of their location of origin along the apical dendrite. This phenomenon of 'synchrony' across the apical dendrite occurs due to the gradient of HCN (H) channels along the apical dendrite.

H-channels, due to their tendency to resist membrane change, act as an inductor in the membrane. The inductive reactance thus generated counteracts the capacitive reactance of the neuronal membrane and advances the phase of oscillatory inputs. Furthermore, as H-channels are distributed in a gradient with increasing conductance from proximal to distal regions of the apical dendrite (4), they generate a gradient of local 'phase advance' (5). Here, we show that this gradient of phase advance counteracts the distance-dependent delay in propagation of inputs, to normalize the timing of inputs across the stratum radiatum during dendritic integration.



The gradient of H-conductance along the apical dendrite synchronizes the timing of incoming theta frequency inputs at the soma

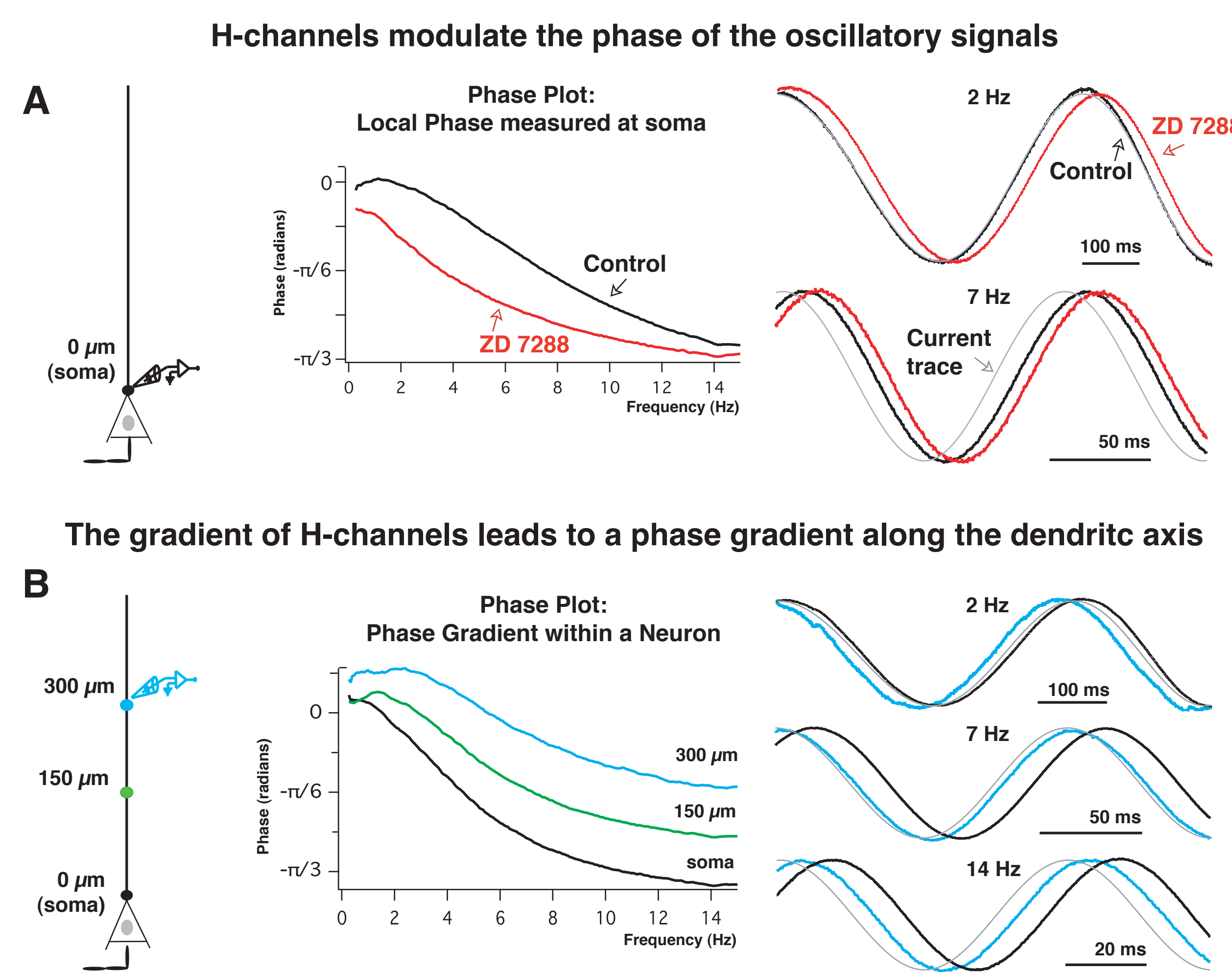


Figure 2: The distribution of H-channels leads to a gradient of local phase advance along the dendritic axis: (A) A somatic recording depicting the ZPP in response to the chirp stimulus before and after the application of HCN (H) channel blocker ZD 7288 (20 μM). The inductive reactance imparted by the h-channels counteracts the capacitive reactance of the neuronal membrane to advance the phase of the voltage response as shown on the right. (B) The gradient of increasing h-conductance from soma to distal dendrites leads to a gradient of increasing local phase advance as shown by the local ZPPs in response to chirps at soma, 150 μm and 300 μm , with corresponding voltage traces on the right.

Theta Frequency Oscillatory inputs from the dendrite and the soma are synchronized at the soma

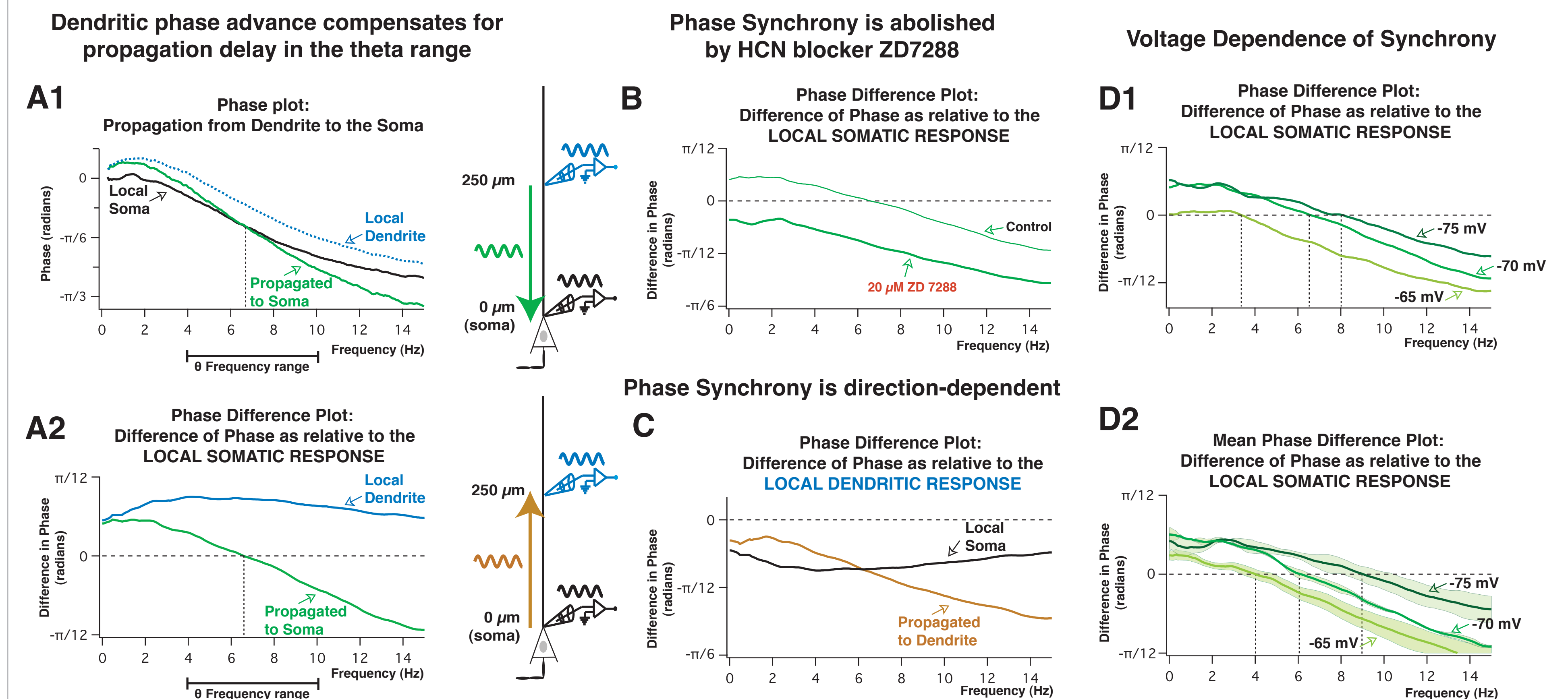


Figure 3: Theta Frequency Oscillatory inputs from the distal dendrite (propagated) and the soma (local) are synchronized at the soma: (A1) ZPPs for chirps injected in the dendrite (250 μm) and the soma, as measured locally (refer adjacent schematic) along with the ZPP for the propagated signal from the dendrite to the soma, as measured at the soma. Note that the propagated ZPP and local somatic ZPP cross in the theta frequency range. This indicates that the propagated oscillatory signal from the dendrite and the local somatic voltage response are coincident at the soma in the theta frequency range. We refer to this as phase synchrony. This occurs as the phase advance of the local dendritic signal compensates for its delay of propagation to the soma. (A2) same as A1, but now, depicted as a difference in phase relative to the local somatic voltage response. (B) shows that the phase synchrony is dependent on the inductive gradient of h-channels, because when blocked with 20 μM ZD7288, the dendritic signal always arrives after the somatic signal irrespective of the input frequency. (C) shows that the phenomenon of phase normalization is directional in nature, as signals propagated in the opposite direction, from the soma to the dendrite, do not show phase synchrony at the dendritic location i.e. oscillatory signals propagated from the soma always arrive after the local dendritic response. (D1) and (D2) show that the frequency of synchronization (as seen in A2) is voltage-dependent for an individual neuron and for a population of neurons, respectively.

Methods

Surgery, slice preparation and Electrophysiology: 10 - 14 week male Sprague Dawley rats were anesthetized with a combination of Ketamine and Xylazine, and transcardially perfused with ice-cold saline solution (with Sucrose replacing NaCl), as per within the guidelines set by the University of Texas iACUC committee. Near horizontal 350 μm thick slices were made and whole cell patch-clamp recordings were made in the current-clamp mode from the middle hippocampal region using an IX-200 dual channel amplifier (Dagan) and a BVC-700 single channel amplifier (Dagan), along with differential interference contrast microscopy on a Nikon Eclipse microscope, fitted with a 60x water-immersion objective lens, for visual guidance.

ACSF contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 2 CaCl_2 , 2 MgCl_2 and 12.5 Dextrose.

Internal pipette solution contained (in mM): 120 K-Gluconate, 20 KCl, 11 HEPES, 4 NaCl, 4 MgATP, 0.3 Na-GTP and 7.2K-phosphocreatinine adjusted to pH 7.35 with KOH.

Pipette Resistance: 4-7 M Ω glass pipettes were pulled fresh before recording and recordings were discontinued if series resistance was above 30M Ω .

Voltages: Although, experiments were carried out at multiple voltages, the data showed here is from traces recorded at -70mV (uncorrected for junction potential, which was approx 8mV). Typically, the neurons rested between -65 and -70 mV

Temperature: 31-33 $^\circ\text{C}$

Impedance profile measurements: 'Chirp' stimulus was used to characterize the impedance profile, which is a sinusoidal current of constant amplitude (50pA for data shown here), linearly spanning 0 - 15 Hz in frequency. The chirp stimulus normally preceded with a 500 ms, 50 pA hyperpolarizing pulse to monitor input resistance.

The magnitude of the ratio of the Fourier transform of the voltage response to the Fourier transform of the Chirp stimulus formed the Impedance Amplitude Profile (ZAP) as follows:

$$|Z(f)| = \sqrt{(\text{Re}(Z(f)))^2 + (\text{Im}(Z(f)))^2}$$

The magnitude of the ratio of the Fourier transform of the voltage response to the Fourier transform of the Chirp stimulus formed the Impedance Phase Profile (ZPP) as follows:

$$\alpha(f) = \tan^{-1} \frac{\text{Im}(Z(f))}{\text{Re}(Z(f))}$$

Local oscillatory dynamics vary with distance from the soma

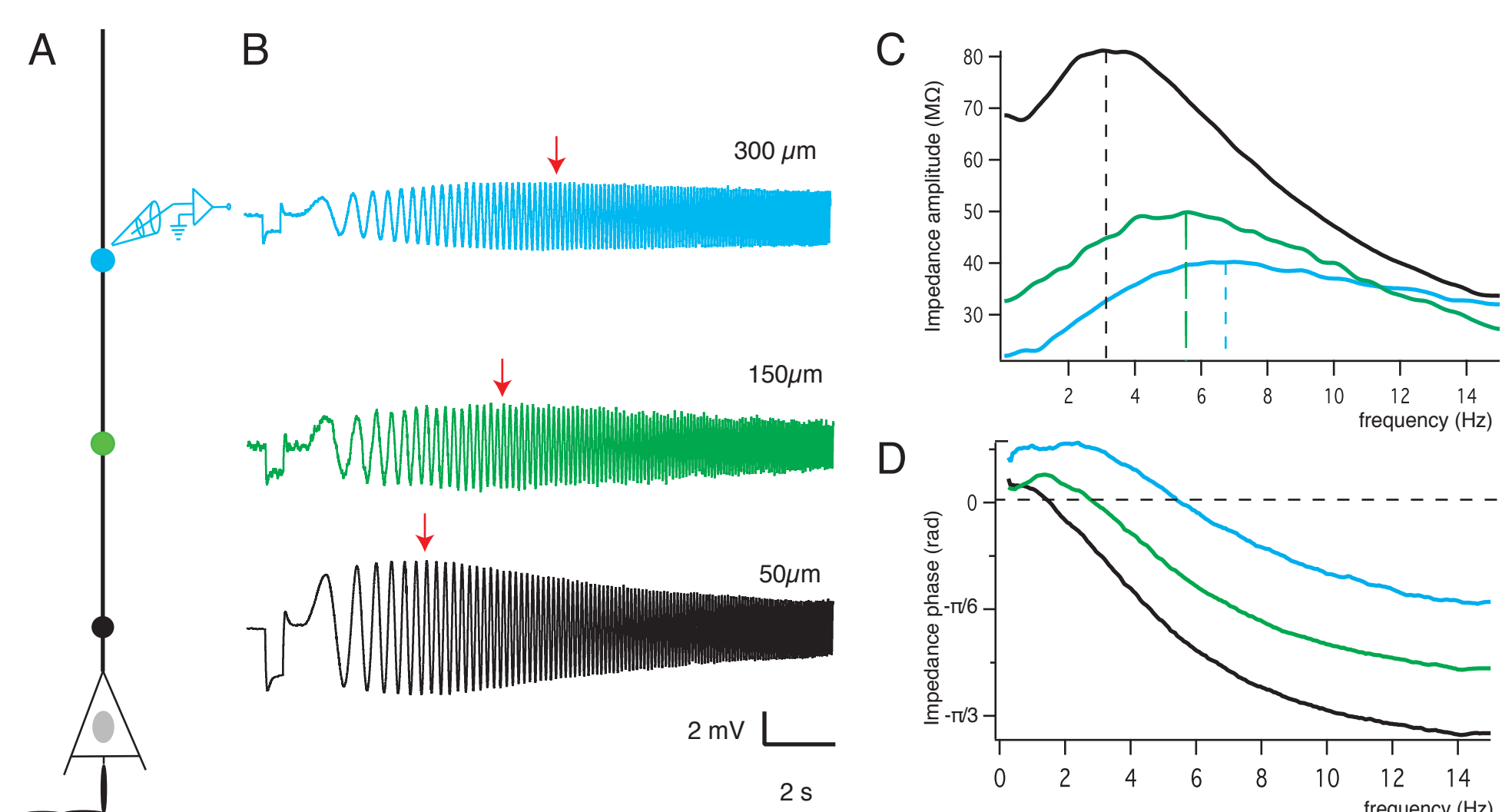


Figure 1: The local impedance measurement along the apical dendrite of a CA1 neuron shows a gradient of increasing resonance frequency and a gradient of phase advance with distance from the soma: (A) Illustration of the apical dendrite of a CA1 neuron along with color-coded distance markers for local measurement of input impedance. (B) The local voltage responses to the chirp stimulus (see methods) used for analysis of impedance amplitude, as depicted in (C), and impedance phase (ZPP), as depicted in (D).

Inputs across the entire apical dendrite are synchronized at the soma for Theta frequency inputs

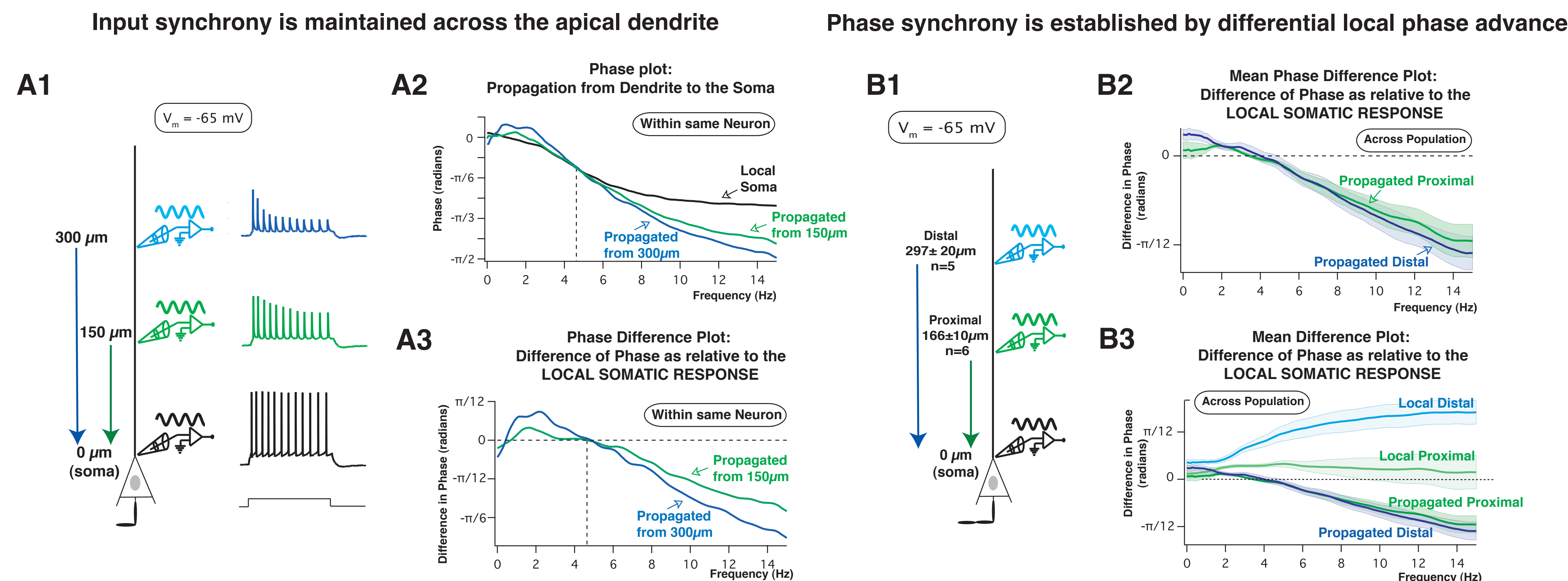


Figure 4: Theta Frequency Oscillatory inputs are synchronized across the apical dendrite at the soma: We performed simultaneous triple whole cell recordings on the soma, proximal dendrite (150 μm) and distal dendrite (300 μm) to investigate if the phase of the propagated oscillatory inputs is synchronized across the apical dendrite at the soma. (A1) depicts the experimental setup along with a train of backpropagating action potentials as measured at the soma, 150 μm and 300 μm . (A2) shows the ZPPs at the soma for chirps injected at 300 μm , 150 μm and the soma. Note, that they have a single crossover point in the theta frequency range. This indicates that irrespective of the input location of the chirp along the apical dendrite, the theta frequency inputs are co-incident at the soma. This synchrony is clearer in the phase difference plot (A3), where the phase differences between proximally and distally propagated inputs, as relative to the somatic response, cross over precisely at the zero line. This phenomenon is further confirmed when data from dual recordings is pooled together in two bins based on the distance (B1) with proximal defined as 150 - 200 μm (166 \pm 10 μm , n=6) and distal as 250 - 350 μm (297 \pm 20 μm , n=5) for testing of phase differences within the propagated signals. As shown in (B2), the average phase differences between proximal and distal propagated inputs are not significantly different near the zero line which occurs in the theta frequency range. This again confirms the hypothesis that irrespective of the origin of the theta frequency oscillatory signal, the propagated responses are phase synchronized at the soma. Moreover, when the local phase differences between the proximal and distal regions are plotted along with the propagated differences (all relative to the somatic local response), it is clear how the neuron exploits the gradient of phase advance to normalize the phase of the propagated inputs at the soma. The distal inputs are significantly phase advanced locally as compared to the proximal inputs (B3), so that they reach the soma at the same time. Thus, the neuron utilizes the gradient of increasing h-channels to normalize the distance-dependent differences in the timing of incoming oscillatory inputs.

The gradient of increasing resonance acts as a band-pass filter for Theta frequency inputs

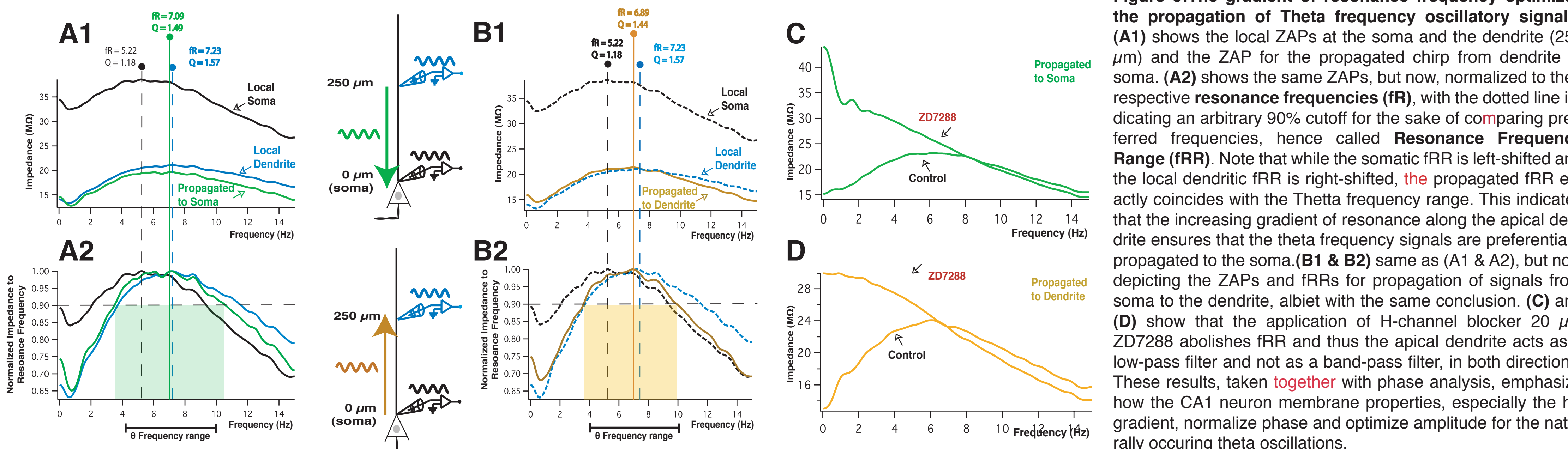


Figure 5: The gradient of resonance frequency optimizes the propagation of Theta frequency oscillatory signals: (A1) shows the local ZAPs at the soma and the dendrite (250 μm) and the ZAP for the propagated chirp from dendrite to soma. (A2) shows the same ZAPs, but now, normalized to their respective resonance frequencies (FR), with the dotted line indicating an arbitrary 90% cutoff for the sake of comparing preferred frequencies, hence called Resonance Frequency Range (FRR). Note that while the somatic FRR is left-shifted and the local dendritic FRR is right-shifted, the propagated FRR exactly coincides with the Theta frequency range. This indicates that the increasing gradient of resonance along the apical dendrite ensures that the theta frequency signals are preferentially propagated to the soma. (B1 & B2) same as (A1 & A2), but now depicting the ZAPs and FRRs for propagation of signals from soma to the dendrite, albeit with the same conclusion. (C) and (D) show that the application of H-channel blocker 20 μM ZD7288 abolishes FRR and thus the apical dendrite acts as a low-pass filter and not as a band-pass filter, in both directions. These results, taken together with phase analysis, emphasize how the CA1 neuron membrane properties, especially the h-gradient, normalize phase and optimize amplitude for the naturally occurring theta oscillations.

Summary

- The gradient of H-channels along the apical dendrite forms a gradient of local phase advance which counteracts the propagation delay for distal inputs towards the perisomatic integration site.

- This normalizes the timing of inputs across the apical dendrite such that irrespective of the input location of a theta frequency oscillatory signal, it is co-incident at the soma.

- Taken together with the band-filtering of theta signals along the apical dendrite, the CA1 neuron achieves both, phase normalization and amplitude optimization for natural rhythmic inputs in the theta frequency range

Acknowledgements & References

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- References:
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