Active dendrites regulate the spatiotemporal spread of signaling microdomains

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Abstract

Microdomains that emerge from spatially constricted spread of biochemical signaling components play a central role in several neuronal computations. Although dendrites, endowed with several voltage-gated ion channels, form a prominent structural substrate for microdomain physiology, it is not known if these channels regulate the spatiotemporal spread of signaling microdomains. Here, we employed a multiscale, morphologically realistic, conductance-based model of the hippocampal pyramidal neuron that accounted for experimental details of electrical and calcium-dependent biochemical signaling. We activated synaptic N-Methyl-D-Aspartate receptors through theta-burst stimulation (TBS) or pairing (TBP) and assessed microdomain propagation along a signaling pathway that included calmodulin, calcium/calmodulin-dependent protein kinase II (CaMKII) and protein phosphatase 1. We found that the spatiotemporal spread of the TBS-evoked microdomain in phosphorylated CaMKII (pCaMKII) was amplified in comparison to that of the corresponding calcium microdomain. Next, we assessed the role of two dendritically expressed inactivating channels, one restorative (A-type potassium) and another regenerative (T-type calcium), by systematically varying their conductances. Whereas A-type potassium channels suppressed the spread of pCaMKII microdomains by altering the voltage response to TBS, T-type calcium channels enhanced this spread by modulating TBS-induced calcium influx without changing the voltage. Finally, we explored cross-dependencies of these channels with other model components, and demonstrated the heavy mutual interdependence of several biophysical and biochemical properties in regulating microdomains and their spread. Our conclusions unveil a pivotal role for dendritic voltage-gated ion channels in actively amplifying or suppressing biochemical signals and their spatiotemporal spread, with critical implications for clustered synaptic plasticity, robust information transfer and efficient neural coding.

Author summary

The spatiotemporal spread of biochemical signals in neurons and other cells regulate signaling specificity, tuning of signal propagation, along with specificity and clustering of adaptive plasticity. Theoretical and experimental studies have demonstrated a critical role for cellular morphology and the topology of signaling networks in regulating this spread. In this study, we add a significantly complex dimension to this narrative by demonstrating
that voltage-gated ion channels on the plasma membrane could actively amplify or suppress the strength and spread of downstream signaling components. Given the expression of different ion channels with wide-ranging heterogeneity in gating kinetics, localization and density, our results point to an increase in complexity of and degeneracy in signaling spread, and unveil a powerful mechanism for regulating biochemical-signaling pathways across different cell types.

**Introduction**

Microdomains that emerge from spatially constricted spread of biochemical signaling components play a central role in defining several neuronal computations, including compartmentalization of neuronal plasticity and localized targeting of membrane components [1–10]. Theoretical and experimental studies have demonstrated that the spread of these microdomains are regulated by several biophysical and biochemical parameters. These parameters include the concentrations, localization profiles, binding and diffusion constants of the signaling components that are part of the signaling network, the morphological structure of the compartment, network topologies and feedback motifs [1–5,8,11]. Most such studies have considered neuronal dendrites, which form the prominent structural substrate for microdomain spread and physiology, to be passive structures that lack active dendritic conductances. Neuronal electrical signaling and physiology, however, is defined by the presence and plasticity of voltage-gated ion channel conductances, some of which are present at higher densities in the dendrites than at the cell body [9,12–24]. Additionally, consequent to their ability to significantly alter calcium influx into neuronal compartments, these active dendritic conductances are well established as critical regulators of synaptic plasticity profiles [12–14,19,25–35]. Given such pivotal role of active dendrites in determining neuronal physiology and plasticity, the question on whether active dendritic conductances regulate the spatiotemporal spread of signaling microdomains is important and has not been addressed.

To address this question, we employed a multiscale, multicompartmental, morphologically realistic, conductance-based model that accounted for the biophysics of electrical signaling [16–18,36,37] and the biochemistry of calcium handling [25,26,38–40] and downstream enzymatic signaling in a hippocampal pyramidal neuron. We chose the calcium–calmodulin–calcium/calmodulin-dependent protein kinase II (CaMKII)–protein phosphatase 1 (PP1) signaling pathway owing to its critical importance to several forms of neuronal plasticity [41–52], and employed physiologically relevant theta-burst stimulation (TBS) or theta-burst pairing (TBP) protocol [12,20,53–58] to initiate a calcium microdomain through N-Methyl-D-Aspartate receptor (NMDAR) activation at a synapse. We studied the spatiotemporal spread of calcium and other downstream microdomains in a dendritic segment compartmentalized to 2000 compartments, each spanning ~97-nm of length.

Using this setup, we assessed the role of two dendritically expressed inactivating conductances [16,18]—one restorative (A-type K⁺) and another regenerative (T-type Ca²⁺)—and showed that they respectively suppress and enhance the spread of phosphorylated CaMKII through different mechanisms. We also assessed the cross-dependencies of these two channels with other model components, and demonstrated the heavy mutual interdependence of several biophysical and biochemical properties in regulating microdomains and their spread. Our results provide compelling evidence for a critical role of active dendrites in regulating the spatiotemporal spread of signaling microdomains. These conclusions call for a marked rethink of the complexities associated with subcellular signaling networks, with future experiments...
focusing on the role of voltage-gated ion channels in tuning location-dependent signaling specificity and spread, in regulating robust information transfer and efficient encoding of afferent inputs in signaling networks, in regulating clustered plasticity of spatially-adjacent synapses on dendritic branches, and in behavioral state- and activity-dependent changes in such signal propagation.

Results

Neuronal excitability is critically regulated by morphological as well as intracellular channel localization profiles [13–15,19,59–61]. As arbitrary choices for morphological properties and channel parameters would preclude extrapolations of our results to physiology, as a first step, we employed a hippocampal pyramidal neuron reconstruction and systematically matched the electrical properties of this model neuron with their electrophysiological counterparts (Fig 1A–1D). This was especially important because a goal of the study was to assess the dependence of signaling spread on neuronal excitability, therefore necessitating the excitability of the model to match measurements from hippocampal neurons across the somatodendritic arbor. The specific signaling pathway that we chose to assess in this study is depicted in Fig 1E. The rationale behind the choice of this pathway was its critical importance to several forms of neuronal plasticity [41–52]. Additionally, from a physiological standpoint, the spread of the CaMKII microdomain in this signaling pathway directly translates to the spread of neuronal plasticity through phosphorylation of several substrates that include 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptors and several ion channels [4,42,43,51,52,56,62]. As the assessment of microdomains requires the analyses of the spatiotemporal spread of each signaling component across the neuronal structure [4,7,8], we employed partial differential equations to model the reaction kinetics coupled to the diffusion of the individual components in the signaling pathway.

Theta burst stimulation to a synapse evoked localized microdomains of calcium and downstream signaling components

To accommodate the steep spatial decay of calcium, and the consequent requirement for finer spatial discretization when compared to discretization required for electrical simulations [63], we followed the established approach of employing a single oblique dendrite to assess signaling microdomains [8]. Although the analyses associated with signaling microdomains (Fig 2A) was performed in this oblique dendrite, its presence as part of the morphologically realistic electrical model ensured that the branching profiles and channel conductances required for maintaining excitability properties match their experimental counterparts. To accommodate fine spatial discretization, the oblique (of total length 193 μm) highlighted in Fig 2A was compartmentalized to 2000 compartments (making each compartment size to be around 97 nm). With the rest of the somatodendritic arbor discretized to accommodate electrical length constants, this spatial discretization procedure resulted in a total number of 2864 compartments in the entire neuronal structure.

We placed a single synapse containing both AMPA and NMDA receptors, whose kinetics and voltage-dependence properties (of NMDA receptors) were derived from electrophysiological measurements, at the center of this 100-μm region on the oblique. The AMPAR density was set such that the somatic unitary EPSP amplitude was ~0.2 mV, to match with experimental observations [64]. Consistent with our motivations of understanding signaling microdomains that are relevant to plasticity induction in the hippocampus, we stimulated this synapse with the well-established theta burst stimulation (TBS; Fig 2A) protocol that induces synaptic plasticity in hippocampal neurons [53]. As expected, the voltage traces obtained with this
stimulation resulted in temporally summating excitatory postsynaptic potentials (EPSPs) that resulted in local dendritic, but not axosomatic spikes (Fig 2A). Although the recorded voltage traces were not very different across a span of 3 μm on either side of the synapse (Fig 2B), the calcium concentration (consequent to influx through NMDARs at the synapse) displayed...
Figure 2. Theta burst stimulation to a synapse evoked localized microdomains of calcium and phosphorylated CaMKII. (A) Left, The morphology of the model neuron with the highlighted oblique where the synapse was located. All traces and analyses presented in B–G were from this oblique dendrite. Right, The position of the synapse within this oblique is represented in a zoomed version. Also shown is the theta burst stimulus (TBS) protocol where 30 bursts, each made of 5 synaptic stimulation pulses (intra-burst frequency: 100 Hz), were presented at 5 Hz. Across all figures depicting distances from the synapse, to avoid ambiguity, distances towards terminal and trunk are represented as positive and negative numbers, respectively. Inset, Voltage response at the center of the oblique (green) where the synapse is located and simultaneously recorded somatic (black) voltage response to a single burst of the TBS stimulus (100 Hz, five pulses). It may be noted that the local oblique response manifests a dendritic spike (amplitude of voltage response at oblique: 60 mV), which does not propagate to the cell body (amplitude of voltage response at soma: 1 mV) owing to attenuation during its somatodendritic propagation. (B) Voltage responses to TBS recorded at the synaptic location and at different distances, towards the trunk and terminal, from this location. Trace in red: Voltage response to a single burst of synaptic stimulation, which is highlighted in red on the trace recorded at the synaptic location. (C) Cytosolic calcium [Ca]_c traces recorded in response to TBS, at the same locations as that of the voltage response in B. (D–F) Maximum value of cytosolic calcium concentration, [Ca]_c^{max} (D), minimum value of unbound calmodulin concentration, [CaM]^{min} (E) and maximum value of phosphorylated CaMKII concentration, [pCaMKII]^{max} (F) plotted as functions of distance from the synapse. A double exponential fit (of the form \(A + B \exp(-x/\lambda_1) + C \exp(-x/\lambda_2)\)) to the distance-dependent calcium decay in panel (D) resulted in values for calcium space constants of \(\lambda_1 = 0.1 \mu m\) and \(\lambda_2 = 2 \mu m\). (G) Kymograph flowchart of the signaling molecules showing the initiation (the calcium trace) and spatiotemporal propagation of microdomains along the signaling cascade. All parameters and channel conductances were assigned to their default values (Fig 1; Tables 1 and 2). Details of parametric values (of active and passive properties in the oblique dendrite shown in Fig 2A) employed for simulations presented in this figure are provided in S1 Table.

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sharp attenuation with distance, thereby establishing the calcium microdomain induced by TBS (Fig 2C and 2D).
Given the reaction-diffusion framework employed here (Fig 1E), this calcium microdomain propagated along the signaling network, manifesting as localized increases in the concentration of calcium-bound calmodulin, activated and phosphorylated CaMKII (pCaMKII) and as localized reductions in the concentrations of unbound calmodulin and non-activated CaMKII (Fig 2E–2G). As would be expected from the binding kinetics of the reactions, and especially by the autophosphorylation of CaMKII, there was an increase in the spatiotemporal spread of the microdomain associated with pCaMKII compared to that of calcium (compare Fig 2D with Fig 2F; see Fig 2G). These results quantitatively demonstrate that TBS induces localized calcium influx, which, through propagation along an established signaling pathway, results in a microdomain of pCaMKII with a spread larger than that of the calcium microdomain [41,43,47].

In order to look at how several key parameters of the model affect the microdomain spatiotemporal kinetics, we performed sensitivity analyses involving different values of calcium diffusion constant ($D_{Ca}$), total concentrations of calmodulin ($[CaM]_T$) and CaMKII ($[CaMKII]_T$). We observed no significant change in the electrical response to TBS for different values assigned to each of these three parameters (Fig 3A), which was expected because all these parameters are involved in the signaling pathway downstream of electrical responses. However, the spatiotemporal dynamics of downstream signaling microdomains were sensitive to these parametric values (Fig 3A). Specifically, increase in $D_{Ca}$ expectedly enhanced the spatial

### Table 1. Parameters employed in modeling calcium dynamics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>Average amplitude of SERCA pump uptake</td>
<td>$V_{max}$</td>
<td>$10^{-4}$</td>
<td>mM ms$^{-1}$</td>
</tr>
<tr>
<td>Dissociation constant for Ca$^{2+}$ binding to a pump</td>
<td>$K_p$</td>
<td>0.27</td>
<td>μM</td>
</tr>
<tr>
<td>Initial cytosolic Ca$^{2+}$ concentration</td>
<td>$[Ca]_0$</td>
<td>0.05</td>
<td>μM</td>
</tr>
<tr>
<td>Ca$^{2+}$ concentration in ER</td>
<td>$[Ca]_{ER}$</td>
<td>400</td>
<td>μM</td>
</tr>
<tr>
<td>Average rate of Ca$^{2+}$ flux density at the plasma membrane</td>
<td>$\gamma$</td>
<td>8.0</td>
<td>μm s$^{-1}$</td>
</tr>
<tr>
<td>Threshold condition for Ca$^{2+}$ extrusion at plasma membrane</td>
<td>$[Ca]_{crit}$</td>
<td>0.2</td>
<td>μM</td>
</tr>
<tr>
<td>Total static buffer concentration</td>
<td>$[B_s]$</td>
<td>450</td>
<td>μM</td>
</tr>
<tr>
<td>Static buffer dissociation constant</td>
<td>$K_s$</td>
<td>10</td>
<td>μM</td>
</tr>
<tr>
<td>Ca$^{2+}$ diffusion coefficient</td>
<td>$D_{Ca}$</td>
<td>220</td>
<td>μm$^2$s$^{-1}$</td>
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Given the reaction-diffusion framework employed here (Fig 1E), this calcium microdomain propagated along the signaling network, manifesting as localized increases in the concentration of calcium-bound calmodulin, activated and phosphorylated CaMKII (pCaMKII) and as localized reductions in the concentrations of unbound calmodulin and non-activated CaMKII (Fig 2E–2G). As would be expected from the binding kinetics of the reactions, and especially by the autophosphorylation of CaMKII, there was an increase in the spatiotemporal spread of the microdomain associated with pCaMKII compared to that of calcium (compare Fig 2D with Fig 2F; see Fig 2G). These results quantitatively demonstrate that TBS induces localized calcium influx, which, through propagation along an established signaling pathway, results in a microdomain of pCaMKII with a spread larger than that of the calcium microdomain [41,43,47].

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### Table 2. Parameters employed in defining kinetics and diffusion of downstream signaling components.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calmodulin concentration</td>
<td>$[CaM]_T$</td>
<td>1</td>
<td>μM</td>
</tr>
<tr>
<td>Diffusion constant for calmodulin</td>
<td>$D_{CaM}$</td>
<td>4</td>
<td>μm$^2$s$^{-1}$</td>
</tr>
<tr>
<td>Forward reaction rate for calcium-calmodulin binding</td>
<td>$k_{on}^{CaM}$</td>
<td>8.4848</td>
<td>mM$^{-1}$ms$^{-1}$</td>
</tr>
<tr>
<td>Dissociation constant for calcium-calmodulin binding</td>
<td>$K_{CaM}$</td>
<td>1.0001</td>
<td>μM</td>
</tr>
<tr>
<td>Total CaMKII concentration</td>
<td>$[CaMKII]_T$</td>
<td>70</td>
<td>μM</td>
</tr>
<tr>
<td>Diffusion constant for CaMKII</td>
<td>$D_{CaMKII}$</td>
<td>1.6</td>
<td>μm$^2$s$^{-1}$</td>
</tr>
<tr>
<td>Forward reaction rate for CaMCa4-CaMKII binding</td>
<td>$k_{on}^{CaMKII}$</td>
<td>100.004</td>
<td>mM$^{-1}$ms$^{-1}$</td>
</tr>
<tr>
<td>Dissociation constant for CaMCa4-CaMKII binding</td>
<td>$K_{CaMKII}$</td>
<td>0.045</td>
<td>μM</td>
</tr>
<tr>
<td>CaMKII autophosphorylation rate</td>
<td>$k_{Auto}^{CaMKII}$</td>
<td>10</td>
<td>μM</td>
</tr>
<tr>
<td>Total PP1 concentration</td>
<td>$[PP1]_T$</td>
<td>10</td>
<td>μM</td>
</tr>
<tr>
<td>Rate constant for PP1 dephosphorylation of pCaMKII</td>
<td>$k_{PP1}$</td>
<td>1.72</td>
<td>s$^{-1}$</td>
</tr>
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</table>

Note that the diffusion constant for CaMCa4 was set as $D_{CaM}$, and those for CaMKII_CaMCa4 and pCaMKII_CaMCa4 were set as $D_{CaMKII}$.

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spread, also resulting in a reduction in peak calcium concentration (Fig 3A and 3B). Consequently, the enhanced spatial spread of calcium, the pCaMKII microdomains showed an enhanced spread, although there was little increase in peak \([\text{pCaMKII}]_{\text{max}}\) (Fig 3A and 3B). Upon increase in \([\text{CaM}]_T\), as more free calcium was now bound to CaM, there was a small reduction in the peak values of \([\text{Ca}^2+]_{\text{c}}\) (Fig 3A and 3B). As a direct consequence of the larger availability of calcium-bound calmodulin, there was a significant increase in the peak values of \([\text{pCaMKII}]\) and in the spread of pCaMKII (Fig 3A and 3B). Varying the \([\text{CaMKII}]_T\), however,
did not alter the calcium or pCaMKII dynamics significantly across the tested range (Fig 3A and 3B). These results demonstrated that within the specific parametric configurations, the spatiotemporal evolution of pCaMKII was more sensitive to the concentration of calmodulin than on calcium diffusion or on total CaMKII concentration.

The presence of A-type potassium channels suppressed the spread of microdomains across the signaling pathway

The presence and plasticity of A-type potassium (KA) channels in hippocampal neuronal dendrites and their roles in regulating dendritic excitability and synaptic plasticity profiles are well established [16,20–22,25–27,29,30,65–68]. Does the presence of or plasticity in KA channels in hippocampal dendrites alter the spread of microdomains in plasticity-inducing enzymes? To address this question, we performed the simulations described in Fig 2 with different densities of KA channels in the oblique specified in Fig 2A (see S1 Table for a figure-wise catalog of oblique parameters). As would be expected from the ability of KA channels to regulate synaptic and action potential amplitudes [16], we found that the voltage response to TBS was lower when the density of KA channels was increased (Fig 4A, 4D and 4E). This difference in voltage response directly translated to changes in the calcium influx through NMDARs (Fig 4B, 4D, 4F, 4H and 4I), and introduced small changes in the spread of the calcium microdomain, especially towards the terminal (Fig 4D and 4F).

Strikingly, this small increase in the spread of calcium microdomain was significantly amplified with propagation along the signaling pathway (Fig 4D, 4F and 4G). Specifically, although the peak pCAMKII response was not very different across different densities of KA channels (Fig 4G and 4J), the spread of the pCaMKII microdomain showed tremendous enhancement with reduction in the density of KA channels (Fig 4G and 4K).

How sensitive are our conclusions to changes in key model parameters? To answer this question, we performed sensitivity analyses to assess the impact of KA channels on pCaMKII microdomain spread with two-fold increase or decrease in several key parameters (Fig 5). Across different parametric combinations, the peak pCaMKII and its spread were consistently larger with lower values of KA channel density, thereby confirming that our conclusions were not restricted by the choice of default parametric values. The sensitivity analyses also demonstrated that the spread of microdomains was critically reliant on several parameters [8], pointing towards robustness of microdomain spread through degeneracy involving several biochemical and biophysical components [8,69]. Specifically, an increase in synaptic AMPAR (Fig 5A) or NMDAR (Fig 5B) densities, or the calcium diffusion constant (Fig 5E), or the autophosphorylation rate of CaMKII (Fig 5G) or the density of R-type calcium (CaR) channels (Fig 5H) enhanced the spread of pCaMKII microdomain. In contrast, an increase in the rate associated with the plasma membrane calcium pump (Fig 5C) or the $V_{\text{max}}$ of the SERCA pump (Fig 5D) or the total capacity of the calcium buffer (Fig 5F) suppressed the pCaMKII microdomain spread.

Together, these results unveil a pivotal role for dendritic A-type potassium channels in suppressing the spatiotemporal spread of microdomains in plasticity-inducing enzymes.

The presence of T-type calcium channels enhanced the spread of microdomains across the signaling pathway

Low voltage-activated transient T-type calcium (CaT) channels, with their predominant dendritic presence, significantly alter synaptic integration, calcium influx and dendritic spike initiation in hippocampal pyramidal neurons [18,70,71]. Although the diverse roles of CaT channels in regulating neuronal physiology and plasticity have been explored [72–76], it is not known if these...
channels contribute to the spread of signaling microdomains of plasticity-inducing enzymes. Therefore, as a next step, we repeated the simulations described in Fig 2 with different densities of CaT channels in the oblique specified in Fig 2A. We found that the peak local voltage response to TBS was slightly higher at terminal end. Additionally, the spatiotemporal voltage response profile was not significantly different with various densities of CaT channels (Fig 6A and 6E), although the cell entered spontaneous spiking with very high density of these channels (e.g., Fig 6A top panel). Although the peak calcium response was not very different with different densities of CaT channels contribute to the spread of signaling microdomains of plasticity-inducing enzymes.
Fig 5. Sensitivity analyses spanning several model parameters confirmed a role for A-type potassium channels in suppressing microdomain spread. The impact of varying critical model parameter values on AUC (black) and peak values (red) of \([p\text{CaMKII}]_{\text{max}}\) at \(g_{KA} = 0 \text{ S/cm}^2\) (open circles) and \(g_{KA} = 1 \text{ S/cm}^2\) (closed circles). (A–H) The parameters varied were AMPAR permeability (A), NMDAR:AMPAR ratio (B), plasma membrane calcium pump (PMCA) density (C), \(V_{\text{max}}\) of the SERCA pump (D), calcium diffusion constant, \(D_C\) (E), total static buffer concentration (F), CaMKII autophosphorylation rate (G), and R-type calcium channel conductance, \(g_{CaR}\) (H). For simulations in this figure, \(g_{CaT} = g_{h} = 0 \text{ mS/cm}^2\) for the oblique represented in Fig 2A. Except for panel H where the sensitivity to \(g_{CaR}\) was
channels (Fig 6B, 6F and 6H), there was a small increase in the spread of calcium microdomain with increase in CaT-channel density (Fig 6B, 6F and 6I).

Despite the absence of large changes in peak voltage and calcium and pCaMKII (Fig 6C, 6G and 6J) responses at the location of the synapse, we found a significantly large increase in the spread of the pCaMKII microdomain with increase in CaT-channel density (Fig 6C, 6G and 6K). Additionally, and in contrast to the case with KA channels (Fig 4G), this increase in spread was symmetric about the synaptic location, which was a consequence of the manner in which these channels altered the microdomain spread. Specifically, whereas KA channels altered the spread of microdomains by asymmetrically modulating the voltage and calcium responses (Fig 4E and 4F), CaT channels modulated the spread through symmetric changes in calcium spread (Fig 6F) without significant changes in voltage response (Fig 6E).

Next, we performed sensitivity analyses on our model to assess the impact of CaT channels on pCaMKII microdomain spread with two-fold increase or decrease in several key parameters (Fig 7). We found, across different parametric combinations, that the spread of pCaMKII was consistently larger with higher values of CaT channel density, thereby confirming that our conclusions were not restricted by the choice of default parametric values. Results from these sensitivity analyses with reference to individual parameters (Fig 7) corroborated our earlier conclusions (Fig 5) on their specific roles in altering pCaMKII microdomains, also providing further evidence for degeneracy in the spatiotemporal spread of signaling microdomains.

Together, our results provide compelling evidence for a critical role for dendritic T-type calcium channels in enhancing the spatiotemporal spread of microdomains in plasticity-inducing enzymes, brought about by increases in calcium influx through these channels.

**Joint regulation of signaling microdomains by A-type potassium and T-type calcium channels**

As a next step in our analyses, instead of individually varying either KA (Figs 4 and 5) or CaT (Figs 6 and 7) channel densities, we varied both channels together in the oblique to different densities and repeated our simulations to assess the spread of pCaMKII microdomains (Fig 8). We performed these simulations at two different densities of CaR channels to assess the interactions between KA, CaT and CaR channels in regulating microdomains. Although results from this set of simulations were consistent with our overall conclusions that KA and CaT channels respectively suppress and enhance the pCaMKII microdomain spread, the quantitative changes observed were dependent on the other channels present in the dendritic branch. For instance, when CaR channels were absent, the impact of KA channels on pCaMKII and its spread (Fig 8A and 8C) was minimal compared to that when CaR channels were present (Fig 8B and 8D). In contrast, from the same set of figures, it may be noted that the impact of CaT channels on pCaMKII and its spread was minimal when CaR channels were absent than when they were present (especially when KA channels were absent). Together these results unveil a crucial role for active dendritic conductances in regulating the spatiotemporal spread of signaling microdomains. Additionally, in conjunction with the sensitivity analyses presented earlier (Fig 3, Fig 5, Fig 7), our conclusions underscore the heavy mutual interdependence of several biophysical and biochemical properties (that account for synaptic, intrinsic and kinetic parameters of several membrane and cytosolic signaling components) in regulating microdomains and their spatiotemporal spread along a signaling pathway.
Spatiotemporal spread of signaling microdomains when synapse was localized on a spine and in the presence of background synaptic activity

Excitatory synapses typically impinge on a dendritic spine, which has been postulated to form a biochemical compartment for calcium and downstream signaling molecules [4,51,77–87]. In order to study the effect of the presence of spine on the signaling microdomains, we...
Fig 7. Sensitivity analyses spanning several model parameters confirmed a role for T-type calcium channels in enhancing microdomain spread. The impact of varying critical model parameter values on AUC (black) and peak values (red) of \([p\text{CaMKII}]\) at \(g_{\text{CaT}} = 0\) mS/cm\(^2\) (open circles) and \(g_{\text{CaT}} = 10\) mS/cm\(^2\) (closed circles). (A–H) The parameters varied were AMPAR permeability (A), NMDAR:AMPAR ratio (B), plasma membrane calcium pump (PMCA) density (C), \(V_{\text{max}}\) of the SERCA pump (D), calcium diffusion constant, \(D_{\text{Ca}}\) (E), total static buffer concentration (F), CaMKII autophosphorylation rate (G), and R-type calcium channel conductance, \(g_{\text{CaR}}\) (H). For simulations in this figure, the conductance values for the oblique represent those in Fig 2A were: \(g_{\text{Ca}} = g_{\text{KA}} = 0\) mS/cm\(^2\). Except for panel H where the sensitivity to \(g_{\text{CaT}}\) was assessed, the value of \(g_{\text{CaT}}\) used in these simulations was 0 mS/cm\(^2\). All other compartments were assigned to default values of conductances (Fig 1). Details of parametric values (of active dendrites regulate signaling microdomains).
incorporated a spine consisting of a head and a neck on the dendritic shaft (Fig 9A) and recorded the spread of voltage, calcium and the downstream signaling molecules in all the three sections. The AMPAR density in the spine was set such that the somatic unitary EPSP amplitude was ~0.2 mV, to match with experimental observations [64]. This implied that the local spine and dendritic voltages were on the order of tens of mV for unitary EPSPs as well as during a TBS input (Fig 9A), which is consistent with large-amplitude spine and local-dendritic voltages recorded during unitary events [88–91]. We also noted that temporal summation during TBS elicited local dendritic spikes in the immediate vicinity of the synapse (Fig 9A), which does not propagate to the soma owing to attenuation during propagation [21].

Consistent with the role of dendritic spines as biochemical compartments, the calcium concentrations which were on the order of 10s of μM at the spine-head dropped significantly with propagation along the spine neck into the dendritic shaft. Specifically, there was almost a 100× attenuation of the calcium levels at the dendritic shaft when compared to that at the spine head (Fig 9B). Whereas the high concentrations of calcium at the spine head could be attributed to the high surface to volume ratio (SVR) of the spine compartments, the significant fall in propagating calcium was consequent to the calcium off mechanisms (i.e., calcium pumps and buffers) expressed in the spine compartments [77,92]. As a consequence of this significant attenuation, the dendritic shaft calcium concentration for a spine-localized synapse was lower than that when the same synapse (with identical receptors and activation dynamics) was located on the dendritic shaft (Fig 9C). Despite the large reduction in the dendritic calcium concentration as a consequence of spine localization, the corresponding reduction in pCaM-KII was comparatively lower (Fig 9C). Overall, despite quantitative differences, the spatiotemporal spread of all molecular species across the signaling topography within the dendritic shaft with a spine-localized synapse (Fig 9D) was qualitatively comparable to the signaling spread with a dendrite-localized synapse (Fig 2G).

Our analyses thus far were performed in the absence of any background synaptic activity. Would the presence of spontaneous background synaptic activity alter the spatiotemporal spread of microdomains across the dendritic structure? To address this, we incorporated randomly activated balanced excitatory and inhibitory synapses throughout the dendritic arbor, resulting in fluctuating membrane potential dynamics (Fig 10A). We then compared the dynamics of voltage propagation and the spread of signaling molecules, across the spine and the dendritic shaft, in the presence or absence of the background synaptic activity (Fig 10B and 10D). Quantitatively, owing to the predominant dendritic presence of background excitatory synapses (Fig 10A), there was a small increase in the dendritic response voltage and the consequent calcium influx in the presence of a background voltage fluctuation (Fig 10B). Overall, although this small increase in dendritic calcium resulted in a minor enhancement of pCaM-KII levels, the spatiotemporal kinetics of the microdomains and the dynamics of their spread were comparable across the spine and the dendrites, irrespective of the presence or the absence of background activity (Fig 10B–10D).

**Active dendritic regulation of the spatiotemporal spread of signaling microdomains when the synapse was localized on a spine**

How do A-type K⁺ and T-type Ca²⁺ channels present on the dendrite regulate microdomain spread when synaptic stimulation arrives on a spine? To address this, we repeated our
Fig 8. Analyses performed in the presence of both \(T\)-type calcium and \(A\)-type potassium channels confirmed the contrasting roles of these channels in regulating the spatiotemporal spread of signaling microdomains. (A–B) Plots depicting the peak values of \([pCaMKII]_{\text{max}}\) with different \(g_{KA}\) and \(g_{CaT}\) values at \(g_{CaR} = 0 \text{ mS/cm}^2\) (A) and \(g_{CaR} = 100 \text{ mS/cm}^2\) (B). The graphs in the bottom panels represent the data presented in the top panel, but plotted differently. (C–D) Same as A–B, but for AUC of \([pCaMKII]_{\text{max}}\). For simulations in this figure, all conductances (other than the ones specified for the oblique under consideration) across compartments were let to assume their respective default values. The oblique \(g_{h}\) was set to 0 for this set of simulations. Details of parametric values (of active and passive properties in the oblique dendrite shown in Fig 2A) employed for simulations presented in this figure are provided in S1 Table.

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simulations described in Fig 9 with different densities of A-type K\(^+\) (Fig 11) and T-type Ca\(^{2+}\) (Fig 12) channels. We found that dendritic A-type potassium channels suppressed the spatio-temporal spread of pCaMKII microdomains (Fig 11), whereas dendritic T-type calcium...
channels enhanced the spatiotemporal spread of these downstream microdomains (Fig 12). As noted earlier, mechanistically, the A-type K⁺ channels regulate the spread of downstream microdomains by altering the voltage response whereas T-type Ca²⁺ channels act through the calcium influx they mediate, without significantly altering the voltage responses. As a consequence of this and because of the tremendous attenuation associated with calcium concentration when the synapse was localized on the spine (Fig 9), the impact of spine localization of the
synapse was quantitatively different in the additional presence of these two channel subtypes (Fig 11 vs. Fig 12). Specifically, the impact of the additional presence of $A$-type $K^+$ channels on $p$CaMKII spread (Fig 11) was significantly lower compared to that of $T$-type $Ca^{2+}$ channels (Fig 12) when the synapse was localized on the spine, because of the respective indirect vs. direct roles of these channels in altering calcium concentration. Together, these results provide further evidence for active dendritic regulation of the spatiotemporal spread of microdomains in plasticity-inducing enzymes through distinct mechanisms and disparate dependencies on synaptic localization profiles.

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Fig 11. A-type potassium channels suppressed the dendritic spread of signaling microdomains in all species of the signaling network, even when the synapse receiving a plasticity-inducing stimulus was localized on a spine. (A) Kymographs depicting the spatiotemporal spread of microdomains along the biochemical signaling cascade in response to TBS with the spine/shaft $g_{K_A} = 0 \text{ mS/cm}^2$ (left) and $g_{K_A} = 1000 \text{ mS/cm}^2$ (right). (B) Peak voltage, $V_{\text{max}}$ (top), maximum value of cytosolic calcium concentration, $[\text{Ca}^{2+}]_{\text{c}}^{\text{max}}$ (middle) and maximum value of phosphorylated CaMKII concentration, $[\text{pCaMKII}]_{\text{c}}^{\text{max}}$ (bottom) as recorded in response to TBS at the spine-head (left), spine-neck (middle) and dendritic shaft (right) for different spine/shaft $g_{K_A}$ levels. (C) Peak value (top panels) and area under the curve, AUC (bottom panels) of $[\text{Ca}^{2+}]_{\text{c}}^{\text{max}}$ (left) and $[\text{pCaMKII}]_{\text{c}}^{\text{max}}$ (right) plotted against $g_{K_A}$ for spine head (red), spine-neck (blue) and oblique shaft (black). All channel conductances were assigned to their default values (Fig 1). Details of parametric values (of active and passive properties in the oblique dendrite shown in Fig 2A) employed for simulations presented in this figure are provided in S1 Table.
Spatiotemporal spread of signaling microdomains in the presence of additional spines in the active dendritic arbor

Our analyses thus far were limited to the presence of a single synapse-containing spine that was placed at the center of the oblique dendrite. How would the microdomain spread and the impact of active dendrites on such spread be affected by the presence of other spine structures on the same dendritic structure? To directly address this question, we randomly placed spines spread across the dendritic oblique under consideration in various numbers (100, 200, 500 and 1000). Each of these additional spines had the same passive and active properties, as well as

**Fig 12. T-type calcium channels enhanced the dendritic spread of signaling microdomains in all species of the signaling network, even when the synapse receiving a plasticity-inducing stimulus was localized on a spine.**

(A) Kymographs depicting the spatiotemporal spread of microdomains along the biochemical signaling cascade in response to TBS with the spine/shaft $g_{CaT} = 0 \text{ mS/cm}^2$ (left) and $g_{CaT} = 10 \text{ mS/cm}^2$ (right). (B) Peak voltage, $V_{\text{max}}$ (top), maximum value of cytosolic calcium concentration, $[\text{Ca}^{2+}]_{\text{max}}$ (middle) and maximum value of phosphorylated CaMKII concentration, $[\text{pCaMKII}]_{\text{max}}$ (bottom) as recorded in response to TBS at the spine-head (left), spine-neck (middle) and dendritic shaft (right) for different spine/shaft $g_{CaT}$ levels. (C) Peak value (top panels) and area under the curve, AUC (bottom panels) of $[\text{Ca}^{2+}]_{\text{max}}$ (left) and $[\text{pCaMKII}]_{\text{max}}$ (right) plotted against $g_{CaT}$ for spine head (red), spine-neck (blue) and oblique shaft (black). All channel conductances were assigned to their default values (Fig 1). Details of parametric values (of active and passive properties in the oblique dendrite shown in Fig 2A) employed for simulations presented in this figure are provided in S1 Table.

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**Spatiotemporal spread of signaling microdomains in the presence of additional spines in the active dendritic arbor**

Our analyses thus far were limited to the presence of a single synapse-containing spine that was placed at the center of the oblique dendrite. How would the microdomain spread and the impact of active dendrites on such spread be affected by the presence of other spine structures on the same dendritic structure? To directly address this question, we randomly placed spines spread across the dendritic oblique under consideration in various numbers (100, 200, 500 and 1000). Each of these additional spines had the same passive and active properties, as well
as the calcium handling mechanisms, although none of them received any synaptic connections. With one of the distinct configurations with reference to the total number of spines on the oblique dendrite, we stimulated the central synapse-containing spine with the TBS protocol, and compared the spatiotemporal spread of microdomains at various spine densities. As a direct consequence of the overall increase in surface area and the active nature of the membrane, we found that the spatial spread of voltage increased with increase in spine density (Fig 13A and 13B), especially in the oblique dendrite for propagation towards the trunk. As a consequence of the presence of additional spines, similar to the impact of increased dendritic diameter on microdomain spread [8], we observed a dissipation of calcium and pCaMKII microdomains consequent to TBS. Specifically, we noted that the peak values of dendritic [Ca] and [pCaMKII] resulting from TBS progressively decreased, and the corresponding spatial spread gradually enhanced, with systematic increase in spine density (Fig 13C).

How does the presence of active dendritic components alter microdomain propagation in the presence of spines? Do our conclusions on the role of KA and CaT channels change with the incorporation of dendritic spines? To test this, we repeated our analyses presented in Figs 11 and 12 with 1000 additional spines (~5 spines/μm [93]) randomly distributed on the oblique under consideration (Fig 14). We performed these analyses for distinct densities of KA (Fig 14A–14C) and CaT (Fig 14D and 14E) channel densities, with all other parameters (except for the incorporation of spines) remaining the same as the simulations performed to obtain Fig 11 (KA channels) and Fig 12 (CaT channels) respectively. We found our conclusions in the presence of these additional background spines to match with our earlier conclusions, whereby the presence of KA and CaT channels respectively suppressed and enhanced the signaling spread of dendritic calcium and pCaMKII (Fig 14).

Spatiotemporal spread of signaling microdomains in the presence of paired backpropagating action potentials during theta-burst stimulation

Although TBS is a widely used LTP-induction protocol, a more robust version of the protocol involves pairing some or all synaptic stimulations as part of TBS with somatically initiated action potentials. The version of TBS where all stimulations are paired with appropriately timed action potentials has been referred to as theta-burst pairing (TBP), with the backpropagating action potentials invading a significant proportion of the dendritic tree allowing for enhanced calcium influx during the protocol [12,20,54–58]. Would the presence of paired backpropagating action potentials alter our conclusions in terms of the spread of downstream microdomains and on the impact of active dendritic conductances on such spread? To test this, we paired synaptic stimulations within TBS with backpropagating action potentials (Fig 15A), and assessed the impact of such TBP on the spread of downstream microdomains.

As expected from the backpropagation of action potentials, there was a small increase in the voltage responses observed in the oblique dendrite during TBP as compared to responses during TBS (Fig 15B). Although the backpropagating action potential amplitude was large on the dendritic trunk (Fig 1C), the invasion of backpropagating action potentials into obliques was minimal because of several factors including the branching structure of the dendritic arbor where obliques are made of smaller diameters, the expression of A-type K⁺ channels in obliques, and the slow recovery of dendritic sodium channels from inactivation [21,22,36,67,94]. As a consequence of this small increase in voltage response, we found small increases in the calcium and pCaMKII concentrations downstream in the spine and dendritic compartments (Fig 15B). These small changes in signaling concentrations also reflected in the spread across the different dendritic segment, overall suggesting that the impact of paired backpropagating action potentials on microdomain spread in oblique dendrites to be minimal (Fig 15C–15E).
Next, we assessed the specific contributions of the active dendritic conductances in regulating the spread of downstream microdomains with TBS or TBP as the plasticity induction protocol. Specifically, we virtually knocked out (by setting the corresponding conductance to zero, only in the dendritic and spine compartments under consideration) either $A$-type K$^+$ or $T$-type Ca$^{2+}$ channels and reassessed the spread of microdomains across the signaling pathway (Fig 15C–15E). Consistent with our prior observations (Figs 4–8, Figs 11 and 12), we found that

**Fig 13. Impact of spine density on the spatiotemporal spread of dendritic signaling microdomains during TBS.** (A) Kymographs depicting the spatiotemporal spread of microdomains along the biochemical signaling cascade in the presence of 1000 spines incorporated in the chosen dendritic oblique (depicted in Fig 2A). (B) Peak voltage, $V_{\text{max}}$ (top), maximum value of cytosolic calcium concentration, $[\text{Ca}^{2+}]_{\text{c}}$ (middle) and maximum value of phosphorylated CaMKII concentration, $[\text{pCaMKII}]_{\text{c}}$ (bottom) as recorded at the spine-head (left), spine-neck (middle) and dendritic shaft (right) for different spine densities. (C) Peak value (top panels) and area under the curve, AUC (bottom panels) of $[\text{Ca}^{2+}]_{\text{c}}$ (left) and $[\text{pCaMKII}]_{\text{c}}$ (right) plotted against number of additional spines that were distributed across the dendritic branch. These values are shown for spine head (red), spine-neck (blue) and oblique shaft (black). All head and neck data shown in this figure correspond to the central synapse-containing spine. All channel conductances were assigned to their default values (Fig 1). Details of parametric values (of active and passive properties in the oblique dendrite shown in Fig 2A) employed for simulations presented in this figure are provided in S1 Table.

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Fig 14. Impact of incorporating dendritic spines on the spatiotemporal spread of signaling microdomains during TBS. (A) Kymographs showing the spatiotemporal spread of voltage (top) and calcium (middle) and pCaMKII (bottom) microdomains in the spine head, spine neck and dendritic shaft in the presence of 1000 spines on the chosen dendritic oblique, plotted with $ \bar{g}_{KA} = 0 \text{ mS/cm}^2$ (left) and $ \bar{g}_{KA} = 1000 \text{ mS/cm}^2$ (right). (B) Peak voltage, $V_{\text{max}}$, maximum value of cytosolic calcium concentration, $[\text{Ca}^{2+}]_{\text{c,max}}$, and maximum value of phosphorylated CaMKII concentration, $[\text{pCaMKII}]_{\text{max}}$, as recorded at the spine head (left), spine neck (center) and dendritic shaft (right) for different values of spines/shaft $ \bar{g}_{KA}$ in the presence of 1000 spines. (C) Peak value (top panels) and area under the curve, AUC (bottom panels) of $[\text{Ca}^{2+}]_{\text{c,max}}$ (left) and $[\text{pCaMKII}]_{\text{max}}$ (right) plotted as a function of spines/shaft $ \bar{g}_{KA}$ for spine-head (red), spine-neck (blue) and oblique shaft (black). (D) Kymographs showing the spatiotemporal spread of voltage (top) and calcium...
knocking out A-type K⁺ or T-type Ca²⁺ channels respectively enhanced and reduced the peak values and the spread of the calcium and pCaMKII microdomains (Fig 15 C–15E). The small differences between TBP and TBS observed earlier, in terms of TBP eliciting a slightly larger calcium response were reflected in both knockout simulations as well (Fig 15D and 15E).

Together, our results provide compelling evidence for a critical role for dendritic channels in regulating the spatiotemporal spread of microdomains in plasticity-inducing enzymes, effectuated by changes in excitability and/or calcium influx, in a manner that was invariant to several structural and parametric configurations.

Discussion

The prime conclusion of this study is that active dendritic conductances play a critical role in regulating the spatiotemporal spread of microdomains associated with plasticity-inducing kinases. We demonstrated this by employing theta-burst synaptic stimulation to a multiscale multicompartamental model that was biochemically and biophysically constrained by experimental measurements. We studied the impact of two inactivating conductances with predominantly dendritic localization profiles, the restorative KA and the regenerative CaT conductances, and showed that they modulate microdomain spread through two distinct mechanisms. Whereas KA channels regulated the spread of pCaMKII microdomains by altering the voltage response to the theta burst stimulus, CaT channels regulated this spread by modulating the calcium influx consequent to TBS without significantly changing the response voltage. Finally, assessing the cross-interactions of KA, CaR and CaT channels (Fig 8) along with their interactions with key structural, biophysical and biochemical parameters (Fig 3, Fig 5, Fig 7, Figs 9–15), we demonstrated the heavy mutual interdependence of several model components in regulating signaling microdomains. Our conclusions unveil a critical role for active dendrites in regulating the spatiotemporal spread of signaling microdomains associated with subcellular molecular networks. Given the physiologically constrained approach that we have employed in this study, our results are also predictions that could be experimentally tested by measuring pCaMKII [43] after TBS or TBP (to different locations along the somatodendritic arbor) in the presence of pharmacological agents to block different channels. Finally, as several cell types express voltage-gated ion channels, and biochemical signaling strength and spread are ubiquitous in their regulatory capacity, our conclusion that voltage-gated ion channels that are present on the plasma membrane could regulate biochemical signaling strength and spread has implications that are not limited only to neurons. In what follows, we discuss the several implications of our conclusions for neuronal physiology and plasticity, and elucidate potential future directions.

Degeneracy in microdomain spread and its implications for robustness of information transfer in signaling pathways

Our results clearly demonstrate that signaling spread is an active process that is not just governed by cell morphology and the topological motifs and binding kinetics associated with the
signaling network [5,8,95–97], but also by the types, kinetics and localization of ion channels in the dendritic arbor. This active amplification (Fig 6, Fig 11, Fig 15) or suppression (Fig 4, Figs 12–15) of biochemical signals and their spread by dendritic ion channel conductances call

Fig 15. Effect of back propagating action potentials on microdomains. (A) Left, Schematic of the pyramidal neuron showing the point of synaptic stimulation (for TBS at center of the oblique dendrite, shown in blue) and the point of current injection at the soma (for eliciting theta burst firing, TBF, shown in black), together resulting in the theta burst pairing (TBP) protocol. Right, The TBP protocol (1 burst) depicting a 5 ms delay between TBS at the synapse and TBF at the soma. The lower panel shows the corresponding 100 ms time-aligned window of voltage traces recorded from the soma (black), the apical trunk at ~100 μm radial distance (red), at ~200 μm radial distance (orange) and at center of the oblique (blue). (B) Voltage (left panels), cytosolic calcium \([\text{Ca}^2+]\) (middle) and phosphorylated CaMKII, pCaMKII (right) traces recorded at the center of the spine-head (top), at the center of spine-neck (middle), at the center of dendritic shaft (bottom) comparing responses obtained with TBP (green) and TBS (black) protocol. At the right of each 10 s trace is shown a magnified 400 ms window. (C) Kymographs showing the spatiotemporal spread of voltage (top) and calcium (middle) and pCaMKII (bottom) microdomains in the spine head, spine neck and dendritic shaft with the TBP protocol, plotted with baseline levels of \(g_{\text{KA}}\) and \(g_{\text{CaT}}\) (left), with \(g_{\text{KA}}\) knocked out (middle) and \(g_{\text{CaT}}\) knocked out (right). (D) Peak voltage, \(V_{\text{max}}\), maximum value of cytosolic calcium concentration, \([\text{Ca}^2+]_{\text{max}}\) and maximum value of phosphorylated CaMKII concentration, [pCaMKII]_{\text{max}} as recorded at the spine head (top), spine neck (middle) and dendritic shaft (bottom) with TBS or with TBP for baseline, \(g_{\text{KA}}\) knockout and \(g_{\text{CaT}}\) knockout cases. (E) Peak value (1st and 3rd panel) and area under the curve, AUC (2nd and 4th panels) of \([\text{Ca}^2+]_{\text{max}}\) (left two panels) and [pCaMKII]_{\text{max}} (right two panels) plotted against all the cases shown in D, for spine-head (red), spine-neck (blue) and oblique dendritic shaft (black). The legend on the right provides the labels for the X-axes of all graphs in this panel. Except for the knockout simulations where specific conductances were set to zero, all channel conductances were assigned to their default values (Fig 1). Details of parametric values (of active and passive properties in the oblique dendrite shown in Fig 2A) employed for simulations presented in this figure are provided in S1 Table.

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for a marked rethink of the complexities associated with subcellular signaling networks. Specifically, the numbers associated with ion channel subtypes, their auxiliary subunits, their subcellular localization profiles, and their local or global modulation through neuromodulatory substances or activity-dependent plasticity are staggeringly combinatorial [9,13–15,23]. The additional regulatory capacity of complex dynamics of subcellular signaling by this complex channel network implies a manifold increase in the complexity of molecular signal transduction. These results also clearly demonstrate several disparate combinations of neuronal parameters—associated with morphology, background spine density, signaling motifs, binding kinetics, diffusion and ion channel densities, for instance—could result in similar signal propagation in a given signaling network, pointing directly to degeneracy in spatiotemporal spread of signaling components [98]. In conjunction with recent literature on degeneracy in cellular-scale physiology and plasticity in hippocampal neurons [24,25,39,99–101], these results point to significant degeneracy in hippocampal physiology spanning different scales.

What are the consequences of such combinatorial complexities and degeneracy associated with signaling networks? First, a growing body of established literature that spans several scales of biology has linked complexity and degeneracy as requirements for robustness in biological systems [69,98,102]. In this context, the degeneracy associated with signaling spread could be postulated as a mechanism for achieving robust signaling transfer and spread in the presence of external and internal noise factors. Our demonstration of active suppression or amplification of signaling strength and spread by specific channels provide an additional mechanism by which noise could be selectively suppressed through channels with specific kinetic and voltage-dependence properties. Second, theoretical frameworks at the cellular scale have argued for efficient coding of incoming information [103–105] through ion channel localization and plasticity [14,28,55,106,107], which at the molecular scale has found reflection in terms of maximizing information transfer by matching signaling dynamics to input source statistics [108–111]. The results described here provide a way to unify these two apparently disparate theoretical frameworks (in two different scales) by showing their convergence towards regulation of signal strength and spread. Future studies should endeavor to holistically unify the systems [103–105], cellular [14,28,55,106,107] and molecular [108–110] versions of the efficient coding hypothesis, accounting for input statistics and neuronal response properties at all scales.

Third, tunability of information transfer is a critical requirement in several signaling networks [112,113]. With active conductances modulating signaling strength and spread, it is clear that the specific signal that is transmitted would now be dependent on the postsynaptic channel densities (along the pathway of spatial propagation) as well, and not just on the input stimulus and the signaling motifs, thus providing an additional regulatory mechanism for tuning signaling specificity and spread.

Location-dependence, plasticity and neuromodulation of microdomains and their spread

Given the location-dependent expression profiles of different ion channels, the dependence of signaling spread on active dendritic conductances could directly translate to location-dependence of signaling spread. Specifically, the density of A-type K+ channels is higher in distal dendrites implying a significant suppression of the spread of signaling at distal locations. However, as different channel conductances have very different channel localization profiles and neuronal physiology is an emergent outcome of intricate and complex spatial and kinetic interactions between these different channels [9,13–15,23,39,99,114], the spread of downstream signaling molecules would also be determined by these interactions (Fig 3, Fig 5, Figs 7–15). Further, as several of these channels have non-uniform distributions, our results imply
that the spread of downstream signaling microdomains might not be symmetric with reference
to the synaptic location (the point of origin of the second messenger). Such a scenario provides
a putative mechanism for spatiotemporally steering the spread and specificity of downstream
signaling by regulating ion channel properties and localization profiles. Finally, given that obli-
que dendritic branches could have different branch strengths as a consequence of differences
in A-type K⁺ channel expression [22], our results present a testable prediction that CaMKII-
dependent plasticity could spread to channels and receptors located over larger distances in
branches with lower A-type K⁺ channel expression (oblique dendrites with higher branch
strength). In branches where the A-type K⁺ channel density is higher, on the other hand, could
have the plasticity confined to a much smaller region owing to the constricted spread of the
CaMKII microdomains. In this context, systematic analyses of the extent of spatiotemporal
influence of different ion channel clusters on signaling microdomains, and of the dependence
of such influence fields on the inhomogeneous distribution of different ion channels, the mor-
phology of the dendritic arbor, the direction of propagation of voltage signals, the presence of
background synaptic activity and the specific location of the channel in the dendritic arbor (e.g.,
on the trunk vs. on the thinner obliques) would provide further quantitative insights into
the roles of active dendrites on the spread of microdomains [114].

Our study presents the possibility of location-dependent expression profiles of channels
and their impact on voltage and calcium signals as potential mechanisms to steer downstream
signaling molecules. However, quantitative links between voltage recordings, calcium tran-
sients and the spatiotemporal spread of downstream microdomains should not be generalized
without specific analyses of the channels and the signaling components expressed in a specific
system. First, although voltage transients provide one trigger for cytosolic calcium influx, they
are not the only source of calcium, with the ER and other store-operated mechanisms playing
a role in regulating calcium influx [26,115–120]. Second, the calcium transients (both ampli-
tude and spread) are critically regulated by several factors (Figs 3–14) including surface-area-
to-volume ratio, spine densities in specific dendritic arbors, the densities of channels, recep-
tors, several pumps, transporters and buffer concentrations [26,77,79,81,87,92,121]. Therefore,
factors such as altered surface-area-to-volume ratio and nonhomogeneous distribution of any
of these components would critically affect calcium amplitude and spread, and alter calcium
transients and downstream signaling independent of changes in voltage transients [100].
Third, the signaling spread of downstream molecular species is not a simple function of voltage
and calcium transients, but is also critically dependent on several factors including surface-
area-to-volume ratio of the compartment, background spine densities, the binding affinities,
diffusion and subcellular localization of the different signaling components, the topology of
the signaling cascade and on the presence of negative regulators upstream [2,4,8,11]. Finally,
all these components—the ones that govern the voltage and calcium transients and those that
govern the downstream signaling—critically interact with each other through several routes
(Fig 3, Fig 5, Figs 7–15), implying a complex parametric and interactional landscape created
by the presence of active dendritic components in regulating signaling microdomains.

Based on these observations, we also postulate that active dendrites constitute a putative
mechanism to regulate clustered plasticity, a phenomenon where spatially adjacent synapses
undergo concurrent plasticity, in dendritic branches [122–127]. Under such a postulate, activ-
ity-dependent plasticity [13–15,19,20,22] and/or state-dependent neuromodulation
[65,72,128,129] of active dendritic conductances could control the degree of compartmentali-
zation of experience-dependent synaptic plasticity on specific dendrites, thereby regulating the
degree of clustering of functional synaptic inputs [123,124,126,127]. Active dendritic conduc-
tances, especially restorative conductances, are therefore very critical in confining the spatio-
temporal spread of plasticity, thereby assigning a dendritic branch as a fundamental functional
unit of biophysical and biochemical signal integration [122–127]. Finally, such regulation of signaling spread by active dendrites, coupled with well-established plasticity and modulation in these dendritic conductances [13–15,19,20,22,65,72,128,129] imply that signaling spread in any signaling molecule in a dendritic branch is dynamic and state-dependent, and that it would be inappropriate to assign a static picture for such a complex dynamical system. This dynamical spread in signaling microdomains has to be assessed accounting for morphological properties of the structure, the network topology, the binding and diffusion kinetics of each signaling component, active dendritic conductances and their properties, the different substrates for the plasticity-inducing enzymes, the localization of all these components, and importantly behavioral state- and activity-dependent modulation and/or plasticity in each of these components.

**Limitations of the analyses and future directions**

In accommodating the significant computational complexity associated with a reaction-diffusion system with stringent requirements on spatial discretization into a morphologically realistic model, we restricted our attention in this study to only a few channel types that are expressed in hippocampal dendrites. Although our analyses provide compelling evidence for a critical role for dendritic ion channels in regulating signaling spread, future studies should systematically characterize the impact of these channel types, including the HCN, calcium-activated potassium and inwardly rectifying potassium channels, on different signaling pathways. Another limitation of our analyses is the absence of metabotropic receptors and calcium-induced calcium release (CICR) and mechanisms associated with the activation of these metabotropic receptors. Specifically, it is established that CICR and other store-related mechanisms significantly interact with plasma membrane ion channels in yielding a complex landscape for the passive and active propagation of calcium within the cytosol [26,115–120]. These CICR mechanisms are critically tied to the activation of specific metabotropic receptors, and have been shown to be involved in certain forms of plasticity through specific signaling cascades [130,131]. Future studies should incorporate these ER-related mechanisms, metabotropic receptors and the complex interactions between dendritic ion channels and ER mechanisms [26,132] in deriving more routes for active-dendritic regulation of signaling microdomains through such interactions.

Although our choice of the signaling pathway was motivated by its physiological relevance to plasticity induction and spread, and by the requirement to reduce computational complexity, the pathway is significantly oversimplified from the standpoint of known complexities in signaling motifs and pathways [4,95–97]. Given the possibility that different ion channels and their spatial and kinetic interactions [39,99,114] could differentially interact with different biochemical network motifs in different morphological structures [7,8], future studies should assess such multiscale interactions towards robustness to internal/external noise, increases in information transmission and storage capacity, efficient encoding of afferent signals and towards tunability of signaling specificity and spread [14,28,39,55,69,98,99,102–114]. From a generic standpoint, the basic conclusion of our analyses on a critical role for voltage-gated ion channels and their structural/functional interactions with the different signaling components in regulating signaling microdomains is extendible to other neuronal structures expressing active dendrites, and even to other cell types expressing voltage-gated ion channels. However, given the critical dependencies on these conclusions on the specific channels and their distributions, on cellular morphologies and on the topology of the signaling cascade, future studies should build cell-specific multiscale models that expand on the analyses presented here. Finally, our reaction-diffusion model is a deterministic system that coupled compartmental
modeling with partial differential equations (of continuous concentrations of signaling species) to assess the spread of microdomains. Although such continuous deterministic models have provided significant insights into biological signal transduction, a more realistic approach to the problem would be to employ a stochastic discrete system (involving number of molecules of signaling species) that would mimic the stochastic interactions of individual molecules within biological systems [4,5,11,133–135].

Methods

We employed a multiscale, multicompartmental, morphologically realistic, conductance-based model that accounted for the biophysics of electrical signaling and the biochemistry of calcium handling and downstream enzymatic signaling in a hippocampal neuron. Parameters associated with these were derived from electrophysiological and biochemical data from hippocampal pyramidal neurons. The biophysical model for electrical signaling and the models for calcium on and off mechanisms, including diffusion, were adapted from previous literature [25,26,38–40]. The signaling pathway, and the biochemical models for enzymatic signaling downstream of calcium were adopted from [10,41–52,97,136–138].

Spatial discretization and passive properties

A morphologically realistic multicompartmental 3D model (Fig 1A) was constructed from a reconstructed CA1 pyramidal neuron morphology (n123) taken from the Neuromorpho database [139,140]. Passive parameters were set as follows: $C_m = 1 \mu F/cm^2$; $R_m$ and $R_a$ for various compartments along the somato-apical trunk were functions of radial distance of the compartment from the soma, $x$ [55]:

$$R_a(x) = R_{a,m} + \frac{(R_{a,end} - R_{a,m})}{1 + \exp\left(\frac{300 - x}{50}\right)} \Omega.cm$$

$$R_m(x) = R_{m,m} + \frac{(R_{m,end} - R_{m,m})}{1 + \exp\left(\frac{300 - x}{50}\right)} k\Omega.cm^2$$

where $R_{m,m} = 125 \text{k}\Omega.cm^2$ and $R_{a,m} = 120 \text{\Omega.cm}$ were values at the soma, and $R_{m,end} = 85 \text{k}\Omega.cm^2$ and $R_{a,end} = 70 \text{\Omega.cm}$ were values assigned to the terminal end of the apical trunk (which was ~425 $\mu$m away from the soma for the reconstruction under consideration). The non-uniformity of passive properties considered here follows from evidence from the literature that has argued for the necessity of such non-uniformity to match electrophysiological measurements [55,114,118,141–143], and has been specifically employed to match passive input resistance of the somato-apical trunk [39]. The basal dendrites, the axonal compartments, and apical obliques had somatic $R_m$ ($R_{m,m} = 125 \text{k}\Omega.cm^2$) and $R_a$ ($R_{a,m} = 120 \text{\Omega.cm}$) [39]. Except for the oblique where the signaling microdomains were assessed (Fig 2A) this neuronal model was compartmentalized using the $d_1$ rule [144] to ensure that each compartment was smaller than 0.1 $\lambda_{100}$, where $\lambda_{100}$ is the space constant, computed at 100 Hz for the section under consideration. This process resulted in 873 compartments for the entire neuronal structure. As eliminating numerical errors in the estimation of $\text{Ca}^{2+}$ signals (whose space constant is on the order of 0.5 $\mu$m) requires much smaller compartment sizes than such electrical compartmentalization [26,63,145], the oblique (of total length 193 $\mu$m) highlighted in Fig 2A was compartmentalized to 2000 compartments (making each compartment size to be around 97 nm). This spatial discretization procedure resulted in a total number of 2864 compartments in the neuronal structure.
**Active conductances across the somatodendritic arbor**

Six different types of voltage gated ion channels (VGIC) were incorporated into these models: a fast Na⁺ (NaF), a delayed rectifier K⁺ (KDR), a hyperpolarization-activated cyclic-nucleotide-gated non-specific cationic (HCN), an A-type K⁺ (KA) and R- (CaR) and T-type Ca²⁺ (CaT). Biophysically realistic, Hodgkin-Huxley type conductance-based models derived from hippocampal pyramidal neurons were employed for modeling all these channels. The kinetics, voltage-dependencies and subcellular localization profiles of these channels were derived from hippocampal pyramidal neurons, and the details are provided below and in S1 Text.

**Sodium and potassium channels.** The densities of NaF and KDR channels were uniform across the somatodendritic arbor [16,18,37]. To account for the lower membrane potential threshold for spike generation at the axon initiation segment, the Na⁺ channel density at this location was increased five fold compared to the somato-dendritic values [146]. The rest of the axon was considered as passive. To account for slower recovery from inactivation of dendritic Na⁺ channels, an extra inactivation gate was added while modeling the channel kinetics for dendritic Na⁺ channels [36,37]. The Na⁺ channel density in apical and basal dendrites was the same as the soma.

\[
\tilde{g}_{Na}(x) = g_{Na}^{\text{som}} \left( 1 + \frac{8x}{100} \right) \text{mS/cm}^2
\]

where \(\tilde{g}_{Na}(x)\) represented the maximal Na⁺ conductance at radial distance \(x\) μm from the soma, and \(g_{Na}^{\text{som}}\) represented the maximal conductance value for Na⁺ channel at the soma, with a default value of 3.1 mS/cm² (translating to 3.1–108.25 mS/cm² over a radial span of 425 μm; Fig 1B). This gradient was set such that the backpropagating action potential amplitude at a trunk location ~300 μm from the soma was around 20 mV [16,37,147]. Reversal potentials for Na⁺ and K⁺ channels were set at 55 and –90 mV, respectively.

**HCN channels.** The kinetics and voltage-dependence of the current through HCN channels were derived from hippocampal pyramidal neuron recordings, with their reversal potential set at ~30 mV [17,148,149]. HCN-channel density [17,55,150] along the somatodendritic arbor was set as (Fig 1B):

\[
\tilde{g}_h(x) = g_h^{\text{som}} \left( 1 + \frac{12}{1 + \exp\left(\frac{320 - x}{50}\right)} \right) \mu\text{S/cm}^2.
\]

This gradient in HCN channel density and the associated passive properties ensured that \(R_m\) decreased from ~65 MΩ at the soma to ~40 MΩ at a trunk location 300 μm away from the soma (Fig 1D). \(g_h^{\text{som}}\), the maximal value of the somatic HCN conductance density was set at 25 μS/cm² (translating to 25–291.68 μS/cm² over a radial span of 425 μm; Fig 1B). The half-maximal activation voltage was set to ~82 mV for compartments <100 μm away from the soma and hyperpolarized linearly up to ~90 mV for compartments up to 300 μm away from the soma, beyond which it stayed at ~90 mV [17,55]. For basal dendrites, HCN-channel density and kinetics were kept the same as those in the somatic compartments, and axonal compartments lacked HCN channels.
Calcium channels. The density of CaT channels increased as a function of distance from the soma, with their kinetics and voltage-dependence derived from hippocampal pyramidal neurons [18,99,151,152].

\[ \bar{g}_{CaT}(x) = \bar{g}_{CaT}^{som} \left( 1 + \frac{30}{1 + \exp((350 - x)/50)} \right) \mu S/cm^2 \]  

(5)

where \( \bar{g}_{CaT} \) represented the density of CaT conductances at the soma, with a default value of 80 \( \mu S/cm^2 \) (translating to 0.08–2.03 mS/cm² over a radial span of 425 \( \mu m \); Fig 1B). In the basal dendrites, CaT channel density was same as that of the soma. CaR channels, when introduced (Figs 4 and 5, Figs 7 and 8), were specific only to the oblique under consideration (Fig 2A), with channel kinetics and voltage dependence derived from hippocampal pyramidal neuron recordings [18,153]. In the baseline model, the CaR channels were absent across the somato-dendritic arbor, and were inserted into the oblique under consideration only to assess the sensitivity of our conclusions to the presence of CaR channels (Figs 4 and 5, Figs 7 and 8). The specific value of \( \bar{g}_{CaR} \) is mentioned in the figure or their legends when it was non-zero. It may be noted that there are several lines of evidence to show that both T- and R-type calcium channels are expressed at higher densities in the dendrites and spines of CA1 pyramidal neurons. First, direct cell-attached recordings from CA1 pyramidal neuron dendrites have shown the high-density expression of these channels in dendritic structures [18]. Second, there are lines of evidence emanating from calcium imaging experiments that point to the expression of R-type calcium channels (CaV2.3) in spines [154,155], and the presence of dendritic T-type calcium channels [71]. Finally, there are also lines of evidence that implicate T-type [73,74] and R-type [74–76] channels as a local calcium source in the induction of plasticity or as a local target of activity-dependent plasticity. The CaT and CaR currents were modeled using the Goldman-Hodgkin-Katz, GHK formulation [156,157] with the default values of external and internal calcium concentrations set at 2 mM and 50 nM, respectively [25,152]. Specifically, the calcium currents through CaT and CaR channels were specified using the following equation [158]:

\[ I_{Ca}(v, t) = \bar{g}_{Ca} S_{Ca}(t) v \left( \frac{1 - \frac{[Ca]_i}{[Ca]_o} \exp \left( \frac{zF}{RT} \right)}{1 - \exp \left( \frac{zF}{RT} \right)} \right) \]  

(6)

where \( S_{Ca}(t) \) represented the product of the activation and inactivation state variables for the respective channel (see S1 Text), \( \bar{g}_{Ca} \) specified the maximal conductance density of the channel (S/cm²), with \([Ca]_i = 50 \text{ nM and } [Ca]_o = 2 \text{ mM.} \)

Glutamate receptors and synaptic stimulation protocol

A canonical glutamate synapse consisting of colocalized NMDARs and AMPARs was placed at the mid point of a proximal oblique represented in Fig 2A. Specifically, the NMDAR current was modeled as a combination of three different types of ionic currents namely Ca²⁺, Na⁺ and K⁺ [29]:

\[ I_{NMDA}(v, t) = I_{NMDA}^{Na}(v, t) + I_{NMDA}^{K}(v, t) + I_{NMDA}^{Ca}(v, t) \]  

(7)
where,

\[ I_{\text{Na}}^{\text{NMDA}}(v, t) = \bar{P}_{\text{NMDA}} P_{\text{Na}} s(t) \frac{vF^2}{RT} \left( \frac{[\text{Na}]_o - [\text{Na}]_i \exp(-\frac{vF}{RT})}{1 - \exp(-\frac{vF}{RT})} \right) \]  

(8)

\[ I_{\text{K}}^{\text{NMDA}}(v, t) = \bar{P}_{\text{NMDA}} P_K s(t) \frac{vF^2}{RT} \left( \frac{[\text{K}]_o - [\text{K}]_i \exp(-\frac{vF}{RT})}{1 - \exp(-\frac{vF}{RT})} \right) \]  

(9)

\[ I_{\text{Ca}}^{\text{NMDA}}(v, t) = \bar{P}_{\text{NMDA}} P_{\text{Ca}} s(t) \frac{4vF^2}{RT} \left( \frac{[\text{Ca}]_o - [\text{Ca}]_i \exp(-\frac{vF}{RT})}{1 - \exp(-\frac{vF}{RT})} \right) \]  

(10)

where \( \bar{P}_{\text{NMDA}} \) is the maximum permeability of the NMDAR; \( P_{\text{Ca}} = 10.6, P_{\text{Na}} = 1, P_K = 1 \) \[159,160\]. Default values of concentrations were (in mM): [Na]_o = 18, [Na]_i = 140, [K]_o = 140, [K]_i = 5, [Ca]_o = 50 \times 10^{-6}, [Ca]_i = 2. The concentrations for sodium set its equilibrium potential at +55 mV and that for potassium at -90 mV. \( MgB(v) \) governs the magnesium dependence of the NMDAR current, given as \[161\]:

\[ MgB(v) = \left( 1 + \frac{[\text{Mg}]_o \exp(-0.062v)}{3.57} \right)^{-1} \]  

(11)

with the default value of [Mg]_o set at 2 mM. \( s(t) \) governed the kinetics of the NMDAR current, and was set as:

\[ s(t) = a \left( \exp\left(-\frac{t}{\tau_d}\right) - \exp\left(-\frac{t}{\tau_r}\right) \right) \]  

(12)

where \( a \) is a normalization constant, making sure that \( 0 \leq s(t) \leq 1 \). \( \tau_d \) is the decay time constant, \( \tau_r \) is rise time, with \( \tau_r = 5 \text{ ms} \), and \( \tau_d = 50 \text{ ms} \).

Current through the AMPAR was modeled as the sum of currents carried by sodium and potassium ions:

\[ I_{\text{AMPA}}(v, t) = I_{\text{AMPA}}^\text{Na}(v, t) + I_{\text{AMPA}}^\text{K}(v, t) \]  

(13)

where,

\[ I_{\text{AMPA}}^\text{Na}(v, t) = \bar{P}_{\text{AMPA}} P_{\text{Na}} s(t) \frac{vF^2}{RT} \left( \frac{[\text{Na}]_o - [\text{Na}]_i \exp(-\frac{vF}{RT})}{1 - \exp(-\frac{vF}{RT})} \right) \]  

(14)

\[ I_{\text{AMPA}}^\text{K}(v, t) = \bar{P}_{\text{AMPA}} P_K s(t) \frac{vF^2}{RT} \left( \frac{[\text{K}]_o - [\text{K}]_i \exp(-\frac{vF}{RT})}{1 - \exp(-\frac{vF}{RT})} \right) \]  

(15)

where \( \bar{P}_{\text{AMPA}} \) is the maximum permeability of the AMPAR, whose default value was set at 20 nm/s, yielding a unitary voltage response of around 0.3 mV. \( P_{\text{Na}} \) was taken to be equal to \( P_K \) \[162\]. \( s(t) \) was the same as that for the NMDA receptor, but with \( \tau_r = 2 \text{ ms} \) and \( \tau_d = 10 \text{ ms} \) \[29\]. \( \bar{P}_{\text{NMDA}} = \text{NAR} \times \bar{P}_{\text{AMPA}} \), with a default value for the NMDAR:AMPAR ratio (NAR) set at 1.5.

We employed theta burst stimulation (TBS), an established protocol for induction of synaptic plasticity \[53\], to assess the spread of signaling microdomains in the model. For TBS, the synapse was stimulated with a burst of 5 action potentials at 100 Hz, and this burst was repeated 30 times at 200 ms interval (5 Hz; theta frequency) each (Fig 2A). To analyze the effect
of back-propagating action potentials (bAPs) initiated during the induction protocol on the spatio-temporal dynamics of microdomains, we used the theta burst pairing (TBP) protocol that has been employed for inducing neuronal plasticity [20,54,55]. In this protocol, each synaptic stimulation pulse during TBS was followed by a current pulse injection at the soma (current clamp amplitude = 2.5 nA; duration of each pulse = 1 ms, each of which initiates an axosomatic action potential that backpropagates into the dendritic arbor as well), with a time lag of 5 ms (Fig 15A). This led to the TBS to be paired with theta-burst firing (TBF), together resulting in the TBP protocol [20,54,55].

### Calcium dynamics

The overall Ca$^{2+}$ dynamics was modeled as a function of various mechanisms that lead to changes in cytosolic Ca$^{2+}$, [Ca]$_c$, within a neuron. The partial differential equation governing the cytosolic Ca$^{2+}$ dynamics was [26,38,163,164]:

$$\frac{\partial [Ca]}{\partial t} = D_{ca} \nabla^2 [Ca] + \beta (J_{\text{leak}} - J_{\text{SERCA}}) + R_{\text{buf}} + J_{\text{CC}} - J_{\text{pump}}$$  \hspace{1cm} (16)

where $D_{ca}$ represents experimentally determined diffusion coefficient for Ca$^{2+}$ [165,166] and $\beta$ constitutes the density of SERCA pumps and leak channels on the ER along the somato-dendritic axis. $J_{\text{CC}}, J_{\text{SERCA}}, R_{\text{buf}}, J_{\text{pump}}$, and $J_{\text{leak}}$ represent the Ca$^{2+}$ flux due to calcium channels, SERCA pumps, buffering, plasma membrane Ca$^{2+}$ extrusion pumps and the ER leak channels, respectively. Radial diffusion of Ca$^{2+}$ was incorporated by radial compartmentalization of the neuronal compartments into 4 concentric annuli, and diffusion along longitudinal axis of the neuron was also implemented [144]. The concentrations of individual molecular species (e.g., calcium, calmodulin) are reported for the outermost annulus of different longitudinal compartments. Detailed descriptions for each of the fluxes are presented below, and a list of all parameters employed for modeling calcium dynamics, with values derived from previous studies [26,38,163,164], are listed in Table 1.

**ER leak channels and SERCA pump.** The rate of Ca$^{2+}$ influx into the cytoplasm through ER leak channels was modeled as [163]:

$$J_{\text{leak}} = \frac{L}{1 - \frac{[Ca]}{[Ca]_c}} \text{mM/ms}$$  \hspace{1cm} (17)

where $L$ was chosen such that there was no net flux of Ca$^{2+}$ between the ER and the cytosol at resting state.

The Ca$^{2+}$ uptake by sarcoplasmic endoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) pump was modeled as [163]:

$$J_{\text{SERCA}} = V_{\text{max}} \frac{[Ca]^2}{[Ca]^2 + K_p^2} \text{mM/ms}$$  \hspace{1cm} (18)

where $V_{\text{max}}$ depicts the maximal rate of pump and $K_p$ is dissociation constant of Ca$^{2+}$ binding to the pump.

**Calcium buffering.** We incorporated buffers uniformly through the somatodendritic axis. $R_{\text{buf}}$ defined the rate of change in [Ca]$_c$ due to buffers:

$$R_{\text{buf}} = -k_{on}[Ca][B_i] + k_{off}[CaB_i]$$  \hspace{1cm} (19)

where $[B_i]$ defined the concentration of stationary buffers, $[CaB_i]$ represented the concentration of Ca$^{2+}$ bound to stationary buffer. Bound buffer was considered to be in rapid equilibrium with the surrounding Ca$^{2+}$ and a pseudo-steady-state approximation was applied to
simulate buffering \[26,38,163,165\]:

\[
\frac{d[B]}{dt} = - \frac{d(CaB)}{dt} = R_{buf}
\]  

(20)

\[
K_s = \frac{k_{on}}{k_{off}}
\]  

(21)

where \(k_{on} = 1000 \text{ /mM-ms}\) and \(k_{off}\) were the on- and off-rate constants for Ca\(^{2+}\) binding to the buffer, and \(K_s = 10 \text{ μM}\) represented the buffer dissociation constant.

**Voltage- and ligand-gated calcium channels.** Ca\(^{2+}\) current through VGCCs (R- and T-type) and through NMDARs was converted to Ca\(^{2+}\) concentration as \[167\]:

\[
J_{cc} = \frac{I_{Ca} \times \pi \times \text{diam}}{300 \times F} \text{mM/ms}
\]  

(22)

where \(I_{Ca}\) depicts the net inward Ca\(^{2+}\) current, \(\text{diam}\) stands for the diameter of the compartment, and \(F\) is Faraday’s constant.

**Plasma membrane calcium extrusion pump.** Ca\(^{2+}\) extrusion through plasma membrane pumps was regulated by a threshold on the \([Ca]_c\). The pumps were inactive below a critical Ca\(^{2+}\) concentration, \([Ca]_{crit}\), above which the extrusion rate depended linearly on \([Ca]_c\) \[163\]:

\[
J_{pump} = \begin{cases} 
\gamma([Ca]_c - [Ca]_{crit}) & : \ [Ca]_c \geq [Ca]_{crit} \\
0 & : \text{otherwise}
\end{cases}
\]  

(23)

where \([Ca]_{crit}\) was set at 0.2 μM based on previous experimental observations \[168\], and \(\gamma\) defines the sensitivity of pump extrusion \[163\].

**Signaling pathway and associated reaction-diffusion processes**

The signaling pathway that was assessed in this study is pictorially represented in Fig 1E \[41–52\]. The specific binding reactions are listed below, with all associated parameters tabulated as Table 2 \[10,97,136–138\]. First, we modeled the binding of cytosolic calcium (Ca) to calmodulin (CaM) to form the calcium-calmodulin complex (CaMCa\(^4\)) as:

\[
4 \text{Ca} + \text{CaM} \Leftrightarrow \text{CaMCa}^4
\]  

(24)

The forward and backward reaction rates were specified as \(k_{on}^{CaM}\) and \(k_{off}^{CaM} \times K_{CaM}\), respectively, with \(K_{CaM}\) defining the dissociation constant related to this binding. Then, the binding of CaMCa\(^4\) to CaMKII (CaMKII) to form a complex (CaMKII_CaMCa\(^4\)) complex was modeled as:

\[
\text{CaMCa}^4 + \text{CaMKII} \Leftrightarrow \text{CaMKII}_C\text{aMCa}^4
\]  

(25)

The forward and backward reaction rates were specified as \(k_{on}^{CaMKII}\) and \(k_{off}^{CaMKII} \times K_{CaMKII}\), respectively, with \(K_{CaMKII}\) defining the dissociation constant related to this binding. Note that CaMKII_CaMCa\(^4\) was alternately represented as CaMKII, with the \(\ast\) representing activation (e.g., Fig 2G). Autophosphorylation of CaMKII was modeled using simple first order kinetics \[45,48,169\] as:

\[
\text{CaMKII}_C\text{aMCa}^4 \longrightarrow p\text{CaMKII}_C\text{aMCa}^4
\]  

(26)

with the associated reaction rate specified as \(k_{Aut}^{CaMKII}\). Note that CaMKII_CaMCa\(^4\) was alternately represented as \(p\text{CaMKII}\), with the \(p\) representing phosphorylation (e.g., Fig 2G).
Finally, the dephosphorylation of CaMKII [50] occurred through protein phosphatase 1 (PP1):

\[
pCaMKII_CaM\text{Ca}4 + PP1 \rightarrow CaMKII_CaMCa4
\]

with the associated reaction rate specified as \(k_{PP1}\). Apart from the radial and longitudinal diffusion of calcium (Eq 16), signaling components represented in Eqs 24–26 also were subjected to radial as well as longitudinal diffusion:

\[
\frac{\partial [X]}{\partial t} = D_X \nabla^2 [X]
\]

where \(X\) represented any of \(CaM, CaMCa4, CaMKII, CaMKII_CaMCa4\) or \(pCaMKII_- \text{CaMCa}4\). As initial conditions, \(CaM\) was set to the total calmodulin concentration, \([CaM]_T\), \(CaMKII\) was set to the total CaMKII concentration, \([CaMKII]_T\), with \(CaMCa4, CaMKII_- \text{CaMCa}4\) and \(pCaMKII_- \text{CaMCa}4\) set to zero. The total concentrations and the diffusion constants for these signaling components are listed in Table 2.

**Measurements and data representation**

Electrical measurements from the model were recorded employing established procedures [39,55] and are detailed below. To measure the backpropagation of action potentials into dendrites [16,39,147], an action potential was initiated at the soma (2 nA current for 1 ms) and the amplitude of the backpropagating action potential (bAP) was measured at various locations along the somatoapical trunk (Fig 1C). Input resistance (\(R_{in}\)) was measured from the local voltage response to a local injection of a 100-pA hyperpolarizing current pulse. The ratio of the steady-state voltage response to the injected current amplitude was taken as the \(R_{in}\) for that location, and the procedure was repeated for all locations along the somatoapical trunk to construct the \(R_{in}\) functional map (Fig 1D).

To avoid ambiguities with reference to distance representations, distance from the synapse toward the terminal and trunk were designated positive and negative values, respectively (Fig 2A and 2B). The spatiotemporal spread of each signaling component was represented as a kymograph with the \(X\)-axis representing time, the \(Y\)-axis representing space (distance from the synapse towards the trunk and terminal), with the color code representing the numerical value of the component that was plotted. The spread of signaling microdomains through the analyzed signal pathway was depicted as a flow chart of kymographs [8]. The extent of the microdomain was quantified as the area under the curve (AUC) of the plot depicting the maximum value of the signaling component as a function of distance from the synapse (e.g., Fig 2F). In computing the AUC for individual compartments, whereas the entire spatial stretch of the compartment was employed for the spine head and spine neck, the dendritic AUC was computed over the span of 50 \(\mu\)m on either side of the synaptic (or spine) location. When the signaling spread was quantified for different parametric configurations, these plots were computed for each parametric configuration (e.g., Fig 4G) and the AUC values obtained from these plots were assessed as a function of the parameter that was being varied (e.g., Fig 4K). With such quantification, an increase or a decrease in the computed AUC would be a measure of enhancement or suppression, respectively, in the spatial spread of the corresponding signaling microdomain. In performing sensitivity analyses with reference to several critical parameters, the default value associated with each parameter was either increased or decreased two-fold (e.g., Fig 5) to assess the impact of such a change in the microdomain spread (quantified as the AUC mentioned above).
Dendritic spine morphology and physiology

For simulations where the synapse was localized on a spine (Figs 9–15), a spine neck (length 1 μm × 0.1 μm diameter) connected to a spine head (length 0.5 μm × 0.5 μm diameter) were added at the center of the oblique dendritic shaft (Fig 9A). The spine-head had 10 compartments whereas the spine-neck had 20 compartments, making the size of each compartment to be ~50 nm. The spine had the same passive and active conductances as that of the center of the oblique dendrite from which it originated: $R_m = 125$ kΩ.cm$^2$, $R_a = 120$ Ω.cm, $g_{Na} = 16$ mS/cm$^2$, $g_{KDR} = 10$ mS/cm$^2$, $g_{KA} = 60.55$ mS/cm$^2$, $g_h = 68.75$ μS/cm$^2$, $g_{CaT} = 285.7$ μS/cm$^2$. A single synapse containing colocalized AMPA and NMDA receptors was placed at the center of the spine head. The AMPAR permeability was set such that the unitary EPSP amplitude at the soma was ~0.2 mV to match experimental observations [64,170,171], and was set at $P_{AMPA} = 15$ nm/s (somatic voltage with spine: 0.22 mV; somatic voltage without spine: 0.25 mV). The NMDAR permeability was set at 1.5× of the AMPAR permeability.

Spine density analysis

To study the impact of spine density on the spatiotemporal spread of biochemical microdomains, we incorporated several spines throughout the oblique dendrite under consideration. Each of these spines had the same morphology as the synapse-containing spine (Fig 9A): spine-neck (length 1 μm × 0.1 μm diameter) connected to a spine-head (length 0.5 μm × 0.5 μm diameter). Each spine had the same passive and active conductances as that of the oblique dendritic compartment from which it originated, including the calcium handling mechanisms. To compare the effect of different densities of spines on the spatiotemporal spread of biochemical microdomains in active dendrites, we compared simulation outcomes in a control (one synapse-containing spine; ~0 spine/μm otherwise) outcomes where the oblique dendrite was populated with spines at four distinct densities. Specifically, the four other cases were built with 100, 200, 500 and 1000 spines (corresponding to ~0.5 spines/μm, ~1 spine/μm, ~2.6 spines/μm and ~5 spines/μm, respectively) distributed randomly (compartments chosen from a uniform distribution) throughout the 2000 compartments of the 193-μm length of the dendritic oblique. All spines except for the synapse-carrying spine (Fig 9A) were devoid of any synaptic connections. With this morphological configuration that reflected the characteristics of a hippocampal pyramidal neuron dendrite, we applied TBS through the central synapse-containing spine and looked at the effects of the spatiotemporal kinetics of each species in our chosen biochemical pathway with different spine densities. We tested the effects of $A$-type K$^+$ channel and $T$-type Ca$^{2+}$ channel densities for the 1000 spine (density = ~5 spines/μm) case, as this spine density represented the closest approximation to experimental evidence [93].

Background synaptic activity

For simulating background synaptic activity impinging on the neuron, we incorporated balanced excitation and inhibition so as to keep the average resting membrane potential (RMP) at around ~65 mV [172]. One excitatory synapse was placed at each compartment of the somatodendritic arbor within a 300-μm radial distance. Similarly, one inhibitory synapse was placed at each compartment within a radial distance of 50 μm perisomatically, including both apical and basal segments. For both the excitatory and inhibitory synaptic populations, independent random spike generators, each firing at an average rate of 4 Hz was used for input stimulation of each synapse. All the synapses were modeled using an Ohmic formulation with the current through the synapse defined as:

$$i_{syn}(t) = g_{syn}(t)(V - E_R)$$  \hspace{1cm} (29)
where \( g_{\text{syn}}(t) \) defined the time-dependent evolution of each synapse after the onset of an afferent spike, and \( E_R \) defined the reversal potential for the synaptic receptors (\( E_R = 0 \) mV for excitatory synapses and \( E_R = -80 \) mV for inhibitory synapses). \( g_{\text{syn}}(t) \) was modeled using a double exponential synaptic formulation:

\[
g_{\text{syn}}(t) = \tilde{g} \left[ \exp\left(-t/\tau_d\right) - \exp\left(-t/\tau_r\right) \right]
\]

where \( \tilde{g} \) defined the maximal conductance of each synapse set at 0.1 nS for excitatory synapses and 0.6 nS for inhibitory synapses. \( \tau_r (= 2 \) ms) was the synaptic rise time constant and \( \tau_d (= 10 \) ms) was the decay time constant for all the synapses. Upon stimulation with such randomized background activity, the mean somatic RMP was found to be -64.33 mV ± 0.74 mV (Fig 10A).

**Computer simulations and analysis**

All simulations were performed in the NEURON simulation environment [144]. The resting membrane potential of the model neuron was fixed at -65 mV. For all experiments, the simulation temperature was set at 34˚C and ion channel kinetics were appropriately adjusted according to their experimentally determined \( Q_{10} \) coefficients. The integration time step was fixed at 25 μs for all simulations to avoid numerical errors in the solution to the differential equations. Data analysis was performed using custom-built software under the IGOR Pro (Wavemetrics Inc., USA) programming environment. The NEURON codes employed to perform the simulations reported in this article are available at the following URL: https://senselab.med.yale.edu/ModelDB/ShowModel.csh.html?model=244848. An updated version of the code in the website fixes and accounts for a volume-scaling bug (See S1 Fig).

All biochemical reactions involving Ca, CaM and CaMKII, their forward rate constants and dissociation constants are listed in S2 Table.

**Supporting information**

S1 Text. Details of kinetic models of ion channels employed in this study. (PDF)

S1 Fig. Outcomes of simulations in an updated model that fixes a volume-scaling bug confirm the role active dendritic conductances in regulating signaling spread. (A) Fig 2D for the updated model, showing maximum value of cytosolic calcium concentration, \([Ca]\)\(_{\text{max}}^c\) plotted as a function of distance from the synapse. (B) Fig 2F for the updated model, showing maximum value of phosphorylated CaMKII concentration, \([pCaMKII]\)\(_{\text{max}}^\text{pl}\) plotted as a function of distance from the synapse. (C) Top, Fig 4H and 4I for the updated model, showing the peak value (left) and area under the curve, AUC (right) of \([Ca]\)\(_{\text{max}}^c\). Bottom, Fig 4J and 4K for the updated model, showing the peak value (left) and AUC (right) of \([pCaMKII]\)\(_{\text{max}}^\text{pl}\). All graphs are plotted against \( \bar{g}_{\text{KA}} \), depicting the suppression of the spread of calcium and pCamKII microdomains by A-type potassium channels. (D) Top, Fig 6H and 6I for the updated model, showing the peak value (left) and area under the curve, AUC (right) of \([Ca]\)\(_{\text{max}}^c\). Bottom, Fig 6J and 6K for the updated model, showing the peak value (left) and AUC (right) of \([pCaMKII]\)\(_{\text{max}}^\text{pl}\). All graphs are plotted against \( \bar{g}_{\text{CaT}} \), depicting the enhancement of the spread of calcium and pCamKII microdomains by T-type calcium channels. All oblique channel parameters are the same as those listed in S1 Table for the specified figures. (TIF)

S1 Table. Table containing values of different channel conductances, in the oblique dendrite shown in Fig 2A, employed for simulations presented in Figs 1–15. (PDF)
S2 Table. Table containing biochemical reactions involving Ca, CaM and CaMKII, their forward rate constants and dissociation constants. (PDF)

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