Active dendrites regulate the impact of gliotransmission on rat hippocampal pyramidal neurons

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An important consequence of gliotransmission, a signaling mechanism that involves glial release of active transmitter molecules, is its manifestation as *N*-methyl-D-aspartate receptor (NMDAR)-dependent slow inward currents in neurons. However, the intraneuronal spatial dynamics of these events or the role of active dendrites in regulating their amplitude and spatial spread have remained unexplored. Here, we used somatic and/or dendritic recordings from rat hippocampal pyramidal neurons and demonstrate that a majority of NMDAR-dependent spontaneous slow excitatory potentials (SEP) originate at dendritic locations and are significantly attenuated through their propagation across the neuronal arbor. We substantiated the astrocytic origin of SEPs through paired neuron–astrocyte recordings, where we found that specific infusion of inositol trisphosphate ($InsP_3$) into either distal or proximal astrocytes enhanced the amplitude and frequency of neuronal SEPs. Importantly, SEPs recorded after $InsP_3$ infusion into distal astrocytes exhibited significantly slower kinetics compared with those recorded after proximal infusion. Furthermore, using neuron-specific infusion of pharmacological agents and morphologically realistic conductance-based computational models, we demonstrate that dendritically expressed hyperpolarization-activated cyclic-nucleotide–gated (HCN) and transient potassium channels play critical roles in regulating the strength, kinetics, and compartmentalization of neuronal SEPs. Finally, through the application of subtype-specific receptor blockers during paired neuron–astrocyte recordings, we provide evidence that GluN2B- and GluN2D-containing NMDARs predominantly mediate perisomatic and dendritic SEPs, respectively. Our results unveil an important role for active dendrites in regulating the impact of gliotransmission on neurons and suggest astrocytes as a source of dendritic plateau potentials that have been implicated in localized plasticity and place cell formation.

neuron–astrocyte interaction | NMDA receptors | HCN channels | transient potassium channels | plateau potentials

Gliotransmission, a signaling mechanism that involves glial release of active transmitter molecules, has been implicated in the regulation of several neurophysiological processes that include synaptogenesis, synaptic transmission and plasticity, neuronal excitability, and synchrony (1–3). An important consequence of gliotransmission is its manifestation as slow inward currents (SIC), events that are mediated by neuronal extrasynaptic *N*-methyl-D-aspartate receptors (NMDAR) and are concomitant to astrocytic calcium elevations (3–8). Although these well-studied events have provided us with important insights into tripartite neuron–astrocyte interactions (1–9), recordings of such SICs have focused predominantly on the soma despite the dendritic localization of a majority of synapses (10). Research over the past two decades has clearly established that dendritic processing critically contributes to neuronal physiology and is best assessed by direct dendritic recordings given the localized nature of such processing (11–15). Importantly, the expression and plasticity of numerous dendritic voltage-gated ion channels (VGIC) and their nontrivial contribution to signal integration have emphasized the need to map neuronal physiology across the somatodendritic arbor (12–18). Juxtaposed against this extensive literature on dendritic physiology, it is surprising that the impact of gliotransmission on neuronal dendrites and the role of dendritic VGICs in regulating this impact have remained unexplored.

Although the role of active dendrites in regulating the impact of synaptic neurotransmission is well established (11–20), direct extrapolation of these results to gliotransmission is impeded by differences between synaptic neurotransmission and extrasynaptic gliotransmission. Specifically, the kinetics of postsynaptic responses to synaptic neurotransmission are much faster compared with the kinetics of such responses to extrasynaptic gliotransmission. As the frequency dependence of passive (14, 21, 22) and active (12, 14, 15, 23, 24) dendritic processing is well established, this difference in kinetics implies that the role of dendrites and their ion channels in altering the impact of gliotransmission could be different from how they alter the impact of neurotransmission. For instance, the spatial spread of the slower gliotransmission could be larger than that of faster neurotransmission as the cable length constant drops significantly with increase in the frequency of the input signal (22), implying a much wider reach of glial-origin signals compared with neurotransmission. Additionally, the density, voltage dependence, and activation/inactivation kinetics of channels in the dendrites confer frequency-dependent processing capabilities, thereby allowing differential processing of slow vs. fast signals (12, 14, 15, 23–26). As several passive and active components and significant interactions among them contribute to such

Significance

Glial cells in the brain actively communicate with neurons through release of transmitter molecules that result in neuronal voltage deflections, thereby playing vital roles in neuronal information processing. Although a significant proportion of information processing in neurons is performed in their dendritic arborization, the impact of gliotransmission on neuronal dendrites has not been mapped. Here, we show that gliotransmission, acting through differentially localized slow receptors, results in strikingly large voltage deflections in neuronal dendrites, with the strength and spread of these deflections critically regulated by dendritic ion channels. Our results add a significantly complex dimension to neuron–glia interactions by demonstrating that neuronal dendrites and their voltage-gated channels play active roles in regulating the impact of such interactions.

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frequency-dependent properties, it is essential to directly assess the role of neuronal dendrites and their ion channels in the strength and spread of the impact of gliotransmission. Finally, as a critical extrasynaptic afferent input that regulates several aspects of neuronal physiology (1–4, 7–9), the extent of the spread of gliotransmitter impact is an important parameter that regulates how these inputs interact and alter synaptic afferents and other ion channels. Specifically, whereas a spatially widespread impact of gliotransmission would imply a lack of distinction between different synaptic afferents and their plasticity, a constriction of the impact of gliotransmission would mean localized computation that involves only adjacent locations within the dendritic structure. Together, despite established differences between the kinetics of neurotransmission and gliotransmission, and despite key differences in the specific physiological roles of gliotransmission that the spatial extent of its impact would imply, a direct and systematic mapping of the impact of gliotransmission on neuronal dendrites is essential but lacking.

To fill these lacunae, we systematically mapped somatodendritic response profiles of slow excitatory potentials (SEP, the voltage counterpart of SIC) mediated by gliotransmitters, using current clamp recordings along the apical dendritic axis and paired neuron–astrocyte recordings in the rat hippocampus. Apart from mapping the spatial dynamics of SEPs, this experimental design allowed us to assess the physiological roles of dendritically expressed channels and receptors in regulating the impact of gliotransmission on neurons. Our results show that dendritic ion channels actively compartmentalize the impact of gliotransmission on neurons to generate localized dendritic plateau potentials and provide evidence for a differential involvement of GluN2B- and GluN2D-containing NMDARs in predominantly regulating perisomatic and distal SEPs, respectively.

Results

As a first step in assessing the spatial dynamics of astrocyte–neuron interactions at the single-neuron scale, we recorded spontaneous SEPs (sSEP) across the somatoapical trunk (up to ∼300 μm away from the soma) of hippocampal pyramidal neurons (in brain slices from 5- to 11-wk-old rats, under physiological temperatures) with synaptic transmission blocked with tetrodotoxin (TTX) and fast AMPA/kainate receptors blocked with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in a magnesium-free extracellular solution. Such systematic mapping would directly address the question of spatial compartmentalization of gliotransmitter impact. Specifically, a spatially compartmentalized spread of sSEPs would reflect as significantly large events around locations of origin with a significant reduction in voltages recorded at other locations. On the other hand, spatially widespread impact of gliotransmission would result in almost equal amplitude signals across the somatodendritic structure. Using direct somatodendritic recordings, we found that dendritic sSEPs were significantly larger in amplitude, with a fourfold increase in the peak amplitudes of sSEPs recorded at distal dendritic locations compared with their perisomatic counterparts (Fig. 1 *A–D* and Fig. S1*E*; perisomatic, 7.4 mV; distal dendrites, 29.5 mV). This increase in sSEP amplitude in distal dendritic locations was accompanied by significant reductions in their rise times and widths (Figs. 1 *E–H* and Fig. S1 *F–G*), together providing evidence for spatially compartmentalized spread of dendritically originating sSEPs. The frequency of these events was comparable with frequency of SICs reported in the literature (7, 27) and did not vary significantly across the somatodendritic axis (Fig. 1*I* and Fig. S1*H*).

Although these results provide functional evidence that a majority of sSEPs originate at dendritic locations, the somatic and dendritic recordings were not from the same neuron. To alleviate this, we performed dual somato-dendritic recordings from the same neuron with identical recording solutions to those above and recorded sSEPs at both a somatic and a dendritic location on the apical trunk (Fig. S2; ∼200 μm away from the soma). Consistent with our previous conclusion on spatial compartmentalization, the amplitudes of sSEPs simultaneously recorded at somatic and dendritic locations were not equal, but exhibited significant variability in how their amplitudes (Fig. S2 *C* and *D*) and kinetics (Fig. S2 *E–G*) were related. We reasoned that such variability should be expected because of the random point of origin of sSEPs that could span the entire dendritic arbor. For instance, if the point of origin of sSEPs were closer to the somatic electrode (say, proximal obliques or basal dendrites), then the amplitude at the somatic electrode would be higher than at the dendritic electrode. To quantitatively evaluate this, we used our computational model (see below) to simultaneously record simulated SEPs at a dendritic (∼200 μm from the soma) and a somatic location, with the point of origin of the SEPs set to span a large portion of the dendritic arbor. We computed the ratio of dendritic amplitude to the corresponding somatic amplitude for SEPs recorded from our dual experiments and our simulations. We found that the distributions of this ratio were not significantly different between experimentally recorded and simulated SEPs (Fig. S2*D*), suggesting that the experimentally recorded SEPs originated from a broad span of the dendritic tree. Taken together, our results (Fig. 1 and Fig. S2) provide direct functional evidence that a majority of sSEPs are not of somatic origin, but originate from a broad span of dendritic locations and undergo significant attenuation through their propagation across the neuronal arbor (Fig. 1 and Fig. S2), pointing to compartmentalization of their spatial spread.

What receptors mediated these sSEPs? Motivated by evidence from the literature that SICs are mediated by NMDA receptors (5–7), we recorded sSEPs in the presence of NMDAR antagonist D,L-2-amino-5-phosphonovaleric acid (D,L APV). Consistent with the literature, we found that these sSEPs were significantly suppressed by APV, in terms of both the amplitude and the frequency of these events (Fig. S3), suggesting that they were mediated by NMDARs. Next, we asked whether these sSEPs were of astrocytic origin by recording neuronal sSEPs after injecting the calcium chelator 1,2-Bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (30 mM) into a nearby astrocyte and allowing it to spread into the astrocytic syncytium for ∼1 h (5, 8). Although such astrocytic calcium chelation elicited complete blockade of sSEPs in one of eight neurons and there was strong reduction in the frequency of events in the others, in some neurons sSEP frequency in the presence of astrocytic BAPTA was comparable to sSEPs recorded in the absence of astrocytic BAPTA (Fig. S4). We noted that these results were consistent with the literature on the suppression of spontaneous SICs recorded after the infusion of BAPTA into the astrocytic syncytium (5, 8).

Dendritic Ion Channels Actively Compartmentalize the Impact of Gliotransmission on Neurons. What mediates the attenuation, filtering, and spatiotemporal compartmentalization of these dendritically originating sSEPs? Hippocampal neuronal dendrites are endowed with a variety of VGICs, expressing with diverse subcellular localization profiles, which mediate several intraneuronal functional maps that significantly contribute to neuronal physiology and plasticity (11–14, 16, 17). Do these active dendritic components play a role in regulating the strength, kinetics, and spread of the impact of gliotransmission on neurons? To answer this, we assessed the role of two prominent VGICs that are expressed at higher densities in the dendrites of these neurons: the *A*-type K^+ (KA) and hyperpolarization-activated cyclic-nucleotide–gated nonspecific-cation (HCN) channels. We found that pharmacological blockade of KA channels, confined to the neuron that was being recorded, resulted in a significant increase in sSEP amplitudes at dendritic locations, but not at the

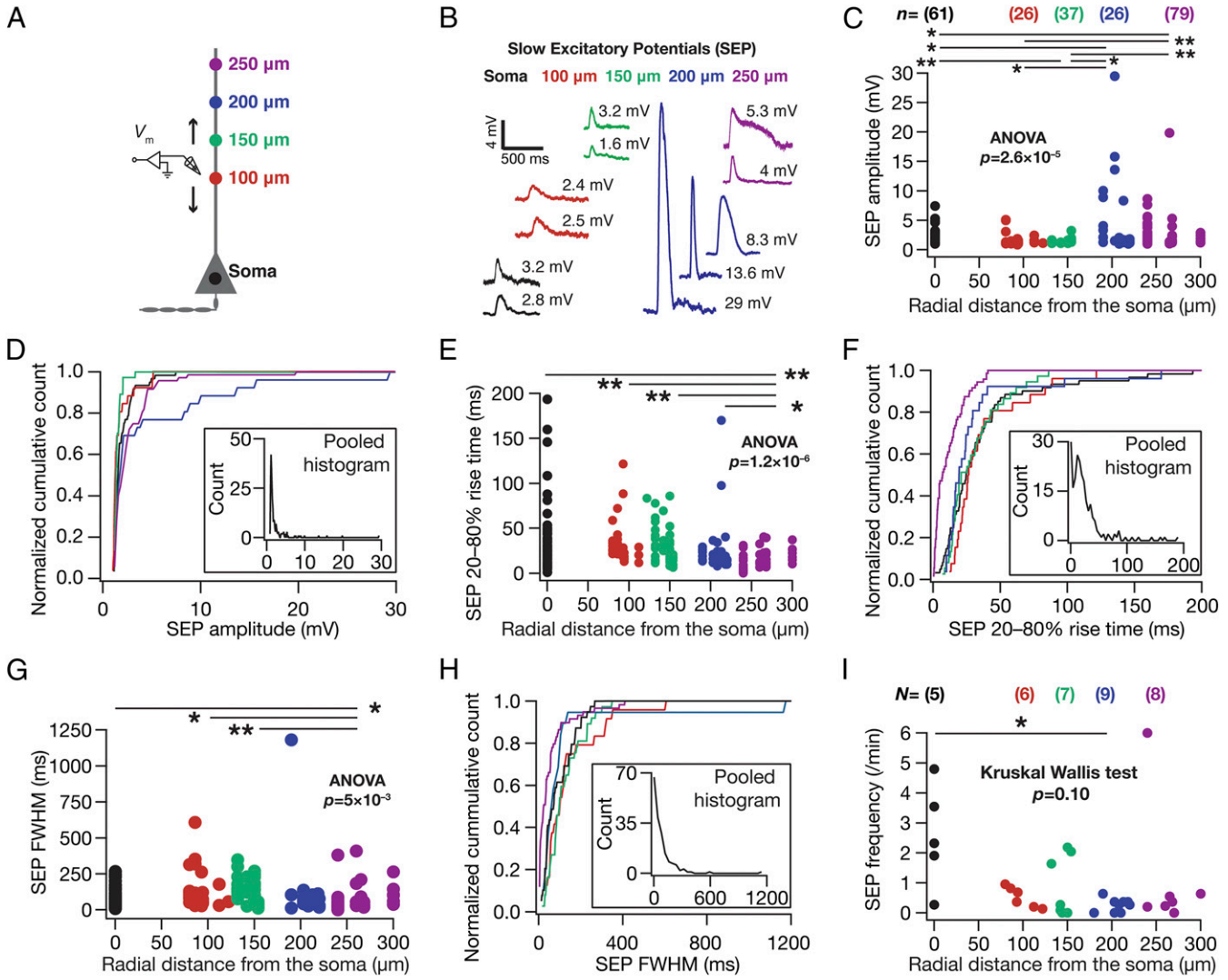

Fig. 1. Spontaneous SEPs recorded at distal dendritic locations were larger in amplitude with faster kinetics than their perisomatic counterparts. (*A*) Schematic of experimental design used to record sSEPs at various locations along the somato-apical trunk, also providing color codes for *B–I*. All data presented in Figs. 1–7 were recorded at physiological temperatures, with synaptic transmission and AMPARs blocked with 1 µM TTX and 10 µM CNQX, respectively. For all figures, *N* represents number of neurons, and *n* depicts number of SEPs. (*B*) Representative traces of sSEPs recorded at different locations along the somato-apical trunk. (*C*) sSEP amplitude as a function of corresponding somato-apical recording location. (*D*) Normalized cumulative histograms of sSEP amplitudes for different locations (data in *C*). *D*, *Inset* depicts a histogram of pooled sSEP amplitudes across all locations. (*E* and *F*) Same as *C* and *D*, for 20–80% rise time of sSEPs. (*G* and *H*) Same as *C* and *D*, for full width at half maximum (FWHM) of sSEPs. (*I*) sSEP frequency for each neuron plotted as a function of recording location. *$P < 0.05$, Mann–Whitney test. For *C*, *E*, and *G*, *$P < 0.05$, **$P < 0.005$, Student's *t* test (Figs. S1–S4).

soma (Fig. 2 *A–D*). However, there was no significant impact of KA-channel blockade on the frequency, rise time, and width of somatic or dendritic sSEPs (Fig. 2 *E–G*). In contrast, pharmacological blockade of HCN channels, confined to the neuron that was being recorded, did not result in significant differences in sSEP amplitudes (Fig. 3 *A–D*). This was despite the emergence of 2 (of 27; Fig. 3*C*) sSEPs that resulted in putative dendritic calcium spikes in the presence of HCN channel blockers. Importantly, blocking HCN channels resulted in significant increases in the rise time, duration, and frequency of dendritic, but not somatic, sSEPs (Fig. 3 *E–G*). Whereas the KA-channel regulation of SEP amplitudes is consistent with their fast kinetics and their ability to regulate synaptic potentials (16), the lack of a significant impact of HCN-channel blockade on SEP amplitudes is consistent with their slow time constants and similar observations on synaptic potentials (17). The positive impact of HCN channels on SEP frequency could be related to the reduced electrotonic distances observed after blockade of these channels (17), implying efficient propagation and improved detectability of propagating SEPs (21, 22). Together, these observations demonstrate that KA and HCN channels critically regulate the amplitude and kinetics of spontaneous SEPs, respectively, and demonstrate that dendritic ion channels actively compartmentalize the impact of gliotransmission on neurons to generate localized excitatory potentials.

Infusion of Inositol Trisphosphate into Proximal or Distal Astrocytes Enhanced the Occurrence and Amplitude of Neuronal SEPs. Although these observations provided direct evidence for a critical role for active dendrites in regulating sSEP amplitudes and compartmentalizing their spread, a significant limitation was the lack of evidence establishing astrocytic origin of these sSEPs. To overcome this, we used a well-established technique of inducing targeted gliotransmitter release from astrocytes through direct infusion of

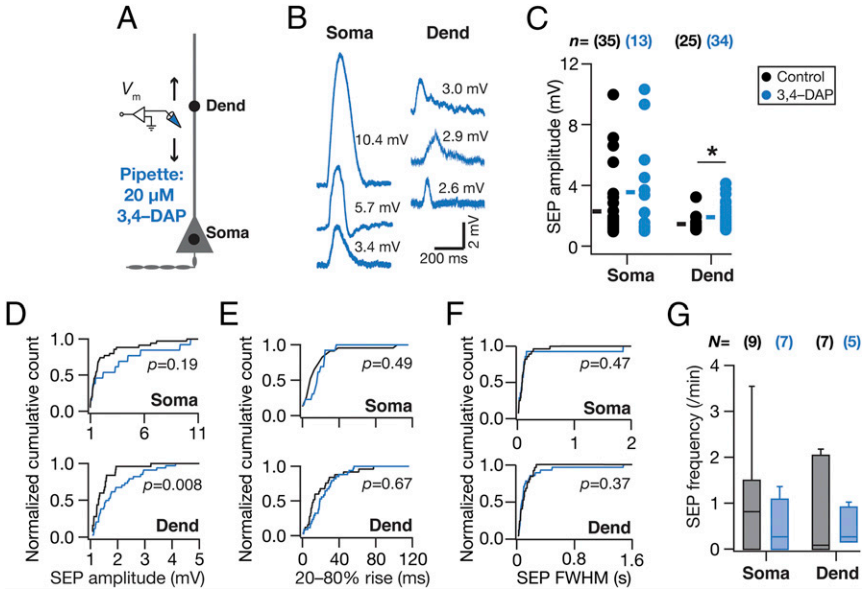

Fig. 2. *A*-type K^+ channels preferentially regulated the amplitude of somatodendritic spontaneous SEPs. (*A*) Schematic of experimental design used to record sSEPs at various locations along the somato-apical trunk with 20 μM 3,4-diamonopyridine (3,4–DAP) in the recording pipette to block KA channels. (*B*) Representative traces of somatic and dendritic sSEPs after blocking KA channels. (*C*) sSEP amplitudes (cyan) recorded at somatic and dendritic (distance from soma for control, 153 ± 5.74 μm; 3,4–DAP = 160 ± 4.47 μm, mean± SEM; $P = 0.36$, Student's *t* test) locations with (3,4–DAP) and without (Control) KA-channel blockade. *$P < 0.05$: Student's *t* test. Rectangles represent the mean values. (*D–F*) Normalized cumulative histograms of sSEP amplitude (*D*), rise time (*E*), and FWHM (*F*) for different locations. *P* values are for Student's *t* test. (*G*) sSEP frequency at somatic and dendritic locations (median and quartiles) with (3,4–DAP) and without (Control) KA-channel blockade.

D-myo-inositol 1,4,5-trisphosphate ($InsP_3$), an endogenous ligand that activated $InsP_3$ receptors on the endoplasmic reticulum, coupled with paired recordings from a nearby neuron (28). We found that infusion of 10 μM $InsP_3$ into visually identified proximal astrocytes (<50 μm away from a patched neuron) significantly increased the peak amplitude and frequency of $InsP_3$-induced SEPs (iiSEPs) in the neuron, compared with SEPs recorded before astrocytic infusion of $InsP_3$ (Fig. 4 and Fig. S5). This increase was specific to infusion of $InsP_3$ because iiSEP amplitudes were significantly higher than sSEP amplitudes recorded in the absence of paired astrocytic recording (Fig. S5) and because large depolarizations of astrocytes in paired recordings, in the absence of $InsP_3$ in the recording pipette, were ineffective in introducing significant differences in recorded SEPs (Fig. S6). Rise time and duration of iiSEPs were not significantly different from their preinfusion conditions in the same neuron (Fig. 4 *E–G*).

Are there differences in iiSEPs evoked through $InsP_3$ infusion into proximal vs. distal astrocytes? As astrocytic processes are well organized with extents of their influence limited to specific regions of their arbor, we performed paired recordings where distal astrocytes (located at around 240 μm along the apical dendritic arbor) were paired with neuronal soma (Fig. 5). Conforming to our results with paired recordings involving proximal astrocytes (Fig. 4), we found that $InsP_3$ infusion into distal astrocytes increased the amplitude and frequency of SEPs recorded in the neuron (Fig. 5 *C–G*). However, the rise time distribution of iiSEPs recorded after $InsP_3$ infusion into distal astrocytes was significantly different from corresponding preinfusion distribution in the same neuron (Fig. 5*H*), consistent with dendritic filtering (22) consequent to predominantly distal origins of these iiSEPs. Concordantly, although the amplitudes (Fig. 5 *J* and *K*) and frequencies (Fig. 5 *L* and *M*) of iiSEPs recorded after $InsP_3$ infusion into proximal (Fig. 4) vs. distal

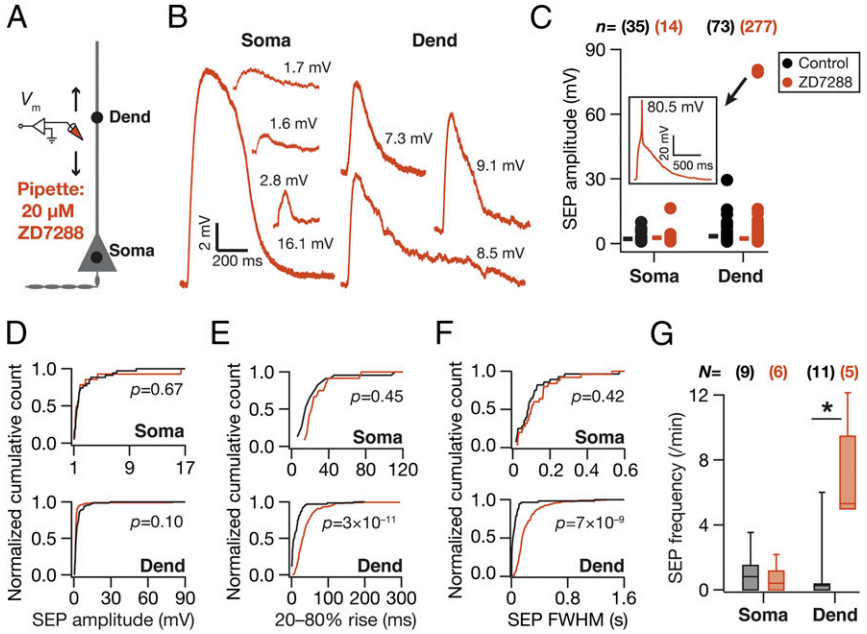

Fig. 3. HCN channels preferentially regulated the kinetics of somatodendritic spontaneous SEPs. (*A*) Schematic of experimental design used to record sSEPs at various locations along the somato-apical trunk with 20 μM 4-Ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride (ZD7288) in the recording pipette to block HCN channels. (*B*) Representative traces of somatic and dendritic sSEPs after blocking HCN channels. (*C*) sSEP amplitudes (cyan) recorded at somatic and dendritic (distance from soma for Control, 213.5 ± 5.6 μm; ZD7288, 212 ± 9.7 μm) (mean ± SEM; $P = 0.9$, Student's *t* test) locations with (ZD7288) and without (Control) HCN-channel blockade. *C*, *Inset* represents a dendritic calcium spike that was induced by the sSEP and rectangles represent the mean values. (*D–F*) Normalized cumulative histograms of sSEP amplitude (*D*), rise time (*E*), and FWHM (*F*) for different locations. *P* values are for Student's *t* test. (*G*) sSEP frequency at somatic and dendritic locations (median and quartiles) with (ZD7288) and without (Control) HCN-channel blockade. *$P < 0.05$, Mann–Whitney test.

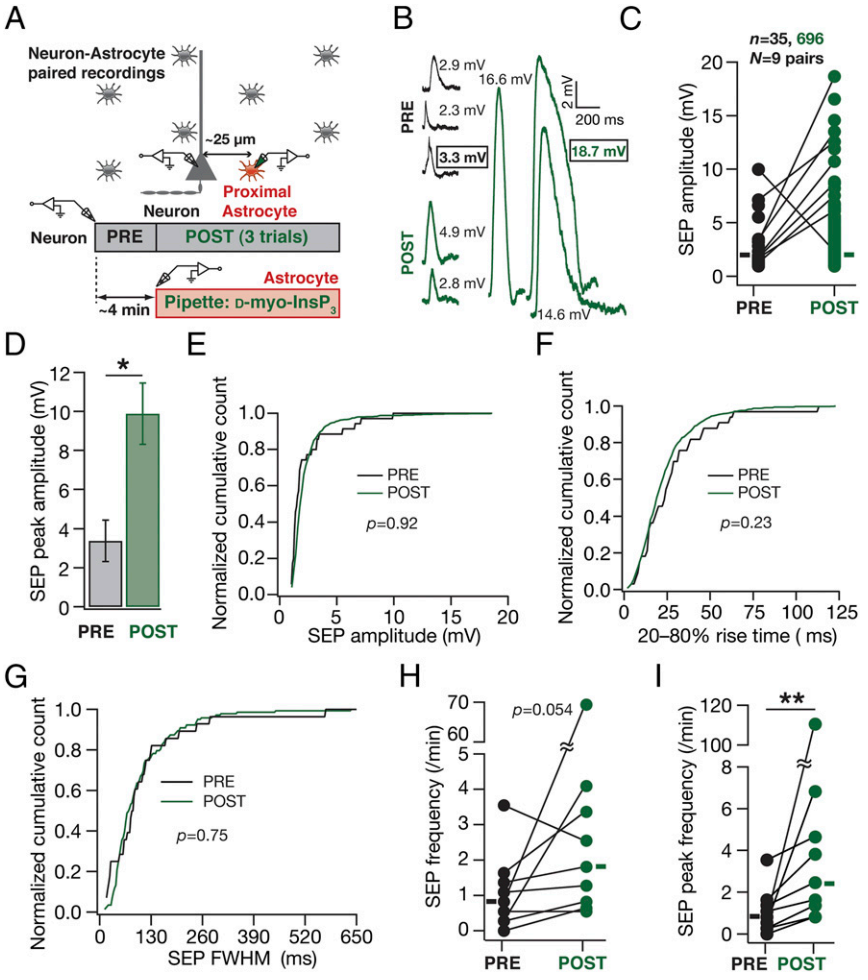

Fig. 4. In paired astrocyte–neuron recordings, infusion of $InsP_3$ into a proximal astrocyte enhanced the occurrence and amplitude of neuronal SEPs. (*A*) Schematic of the experimental design for paired astrocyte–neuron recordings with 10 µM $InsP_3$ in the astrocytic recording pipette. (*B*) Representative traces from a single neuron recorded before (PRE) and after (POST) infusion of $InsP_3$ into a nearby astrocyte. Numbers correspond to SEP amplitudes of adjacent traces, and boxed numbers denote that the adjacent trace exhibited the peak amplitudes recorded during the course of a given experiment (PRE or POST infusion). (*C* and *D*) Amplitude (*C*) and peak amplitude (*D*) of SEPs recorded before (PRE) and after (POST) infusion of $InsP_3$ into a nearby astrocyte. The sticks in *C* connect the peak amplitudes recorded before and after $InsP_3$ infusion in each of the nine pairs and rectangles represent mean values. The plot in *D* depicts a mean ± SEM representation of the PRE and POST peak amplitudes in individual cells. $*P < 0.05$: paired Student's *t* test. (*E*–*G*) Normalized cumulative histograms of amplitude (*E*), 20–80% rise time (*F*), and FWHM (*G*) for SEPs recorded before (PRE) and after (POST) infusion of 10 µM $InsP_3$ into a nearby astrocyte. *P* values are for Student's *t* test. (*H* and *I*) Frequency (*H*) and peak frequency (*I*) of SEPs recorded before (PRE) and after (POST) infusion of $InsP_3$ into a nearby astrocyte. For *H* and *I* rectangles represent the population median values. *P* values are for a paired Mann–Whitney test with $**P < 0.005$ (Figs. S5 and S6).

astrocytes (Fig. 5 *C*–*I*) were not significantly different, the rise time (Fig. 5*N*) and FWHM (Fig. 5*O*) of iiSEPs recorded after $InsP_3$ infusion into distal astrocytes were significantly higher compared with those recorded after proximal infusion.

Together, these observations established that specific activation of an astrocyte through $InsP_3$ infusion significantly enhanced the occurrence and amplitude of SEPs in nearby neurons, thereby providing clear evidence for the astrocytic origin of SEPs. Importantly, we show that iiSEPs recorded after $InsP_3$ infusion into distal astrocytes exhibited significantly slower kinetics compared with those recorded after proximal infusion. These results unveil a direct link between the location of an activated astrocyte and the point of origin of the associated SEP along the somatodendritic axis, thereby further substantiating the astrocytic origin of SEPs.

Blocking KA or HCN Channels Enhanced SEPs Induced by the Infusion of $InsP_3$ into a Nearby Astrocyte. Given the specificity of this paired recording process in delineating the astrocytic origin of SEPs, we returned to our question on the role of neuronal VGICs in regulating the impact of gliotransmission on neurons. Consistent with our results on sSEPs (Figs. 2 and 3), we found that blocking KA channels resulted in an increase in the amplitude of iiSEPs, compared with iiSEPs recorded without KA-channel blockade, without significantly altering their rise time or duration (Fig. 6 *A*–*F*). Additionally, blocking HCN channels resulted in significant increases in the amplitude, rise time, and duration of iiSEPs compared with the conditions where HCN-channel blockers were absent (Fig. 6 *G*–*L*). There were some differences in our results from iiSEPs and sSEPs (e.g., HCN-channel regulation of SEP amplitude or KA-channel regulation of SEP frequency), which could partly be attributed to iiSEPs being larger than sSEPs (Figs. S5, S7, and S8), leading to differential activation of ionic conductances across the somatodendritic arbor. Together, our results from dendritic recordings of spontaneous SEPs (Figs. 2 and 3) and paired neuron–astrocyte recordings of iiSEPs (Fig. 6) clearly demonstrate a critical role for dendritic ion channels in regulating the strength, kinetics, and spread of the impact of gliotransmission on neurons.

GluN2B- and GluN2D-Containing NMDARs Predominantly Mediate Perisomatic and Dendritic SEPs. Previous studies in juvenile rodents have demonstrated SICs to be mediated by GluN2B-containing NMDARs (GluN2BR) (7, 27). However, receptor localization studies in the adult CA1 region have shown GluN2BRs to express predominantly at perisomatic locations (29). In the context of our observations that sSEP amplitudes were larger at dendritic locations (Fig. 1), this receptor localization profile constitutes a paradox. In an effort to resolve this, and motivated by the presence of GluN2D-specific NMDARs (GluN2DR) in the CA1 dendritic region (29), we assessed the specific contributions of GluN2BR and GluN2DR to iiSEPs in paired neuron–astrocyte recordings (Fig. 7*A*). We found that blocking GluN2DR suppressed low-amplitude iiSEPs, whereas blockade of GluN2BR suppressed high-amplitude iiSEPs (Fig. 7 *B* and *C* and Fig. S9*D*). Consequently, whereas blocking of GluN2BR, but not GluN2DR alone, resulted in a significant reduction in the iiSEP amplitude (Fig. 7 *D* and *E*), blocking either

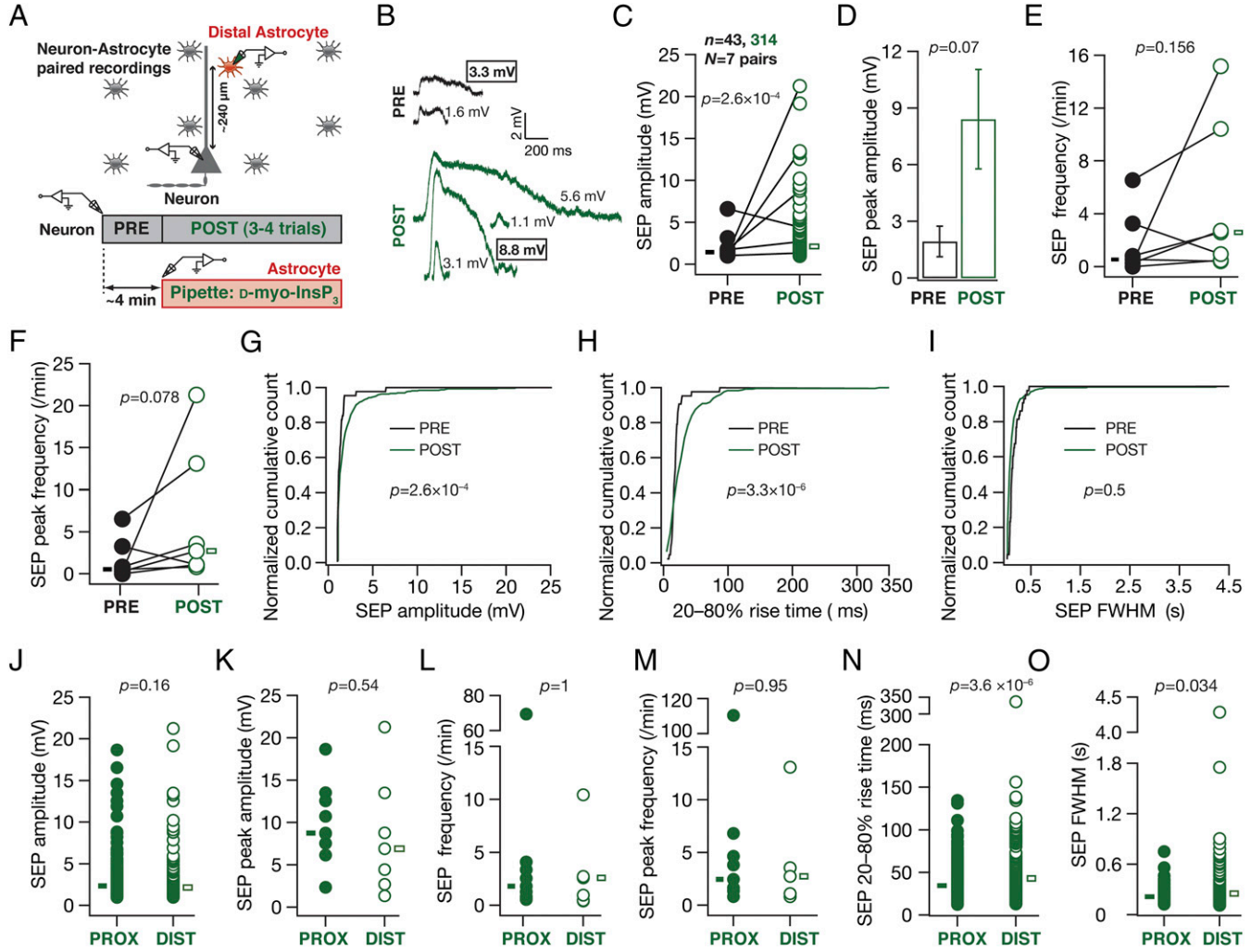

Fig. 5. Infusion of $InsP_3$ into a distal astrocyte enhanced the occurrence and amplitude of neuronal SEPs at the soma, with SEPs exhibiting slower kinetics compared with those recorded with $InsP_3$ infusion into proximal astrocytes. (*A*) Schematic of the experimental design for paired astrocyte–neuron recordings with 10 μM $InsP_3$ in the recording pipette of astrocytes located at a distance of 200–300 μm (240 ± 16.7 μm, mean ± SEM) from the soma in the apical dendritic region. (*B*) Representative traces from a single neuron recorded before (PRE) and after (POST) infusion of $InsP_3$ into a distal astrocyte. Numbers correspond to SEP amplitudes of adjacent traces, and boxed numbers denote that the adjacent trace exhibited the peak amplitude recorded during the course of a given experiment (PRE or POST infusion). (*C* and *D*) Amplitude (*C*) and peak amplitude (*D*) of SEPs recorded before (PRE) and after (POST) infusion of $InsP_3$ into a distal astrocyte. The sticks in *C* connect the peak amplitudes recorded before and after $InsP_3$ infusion in each of seven pairs. Boxes in *C* represent the mean SEP amplitudes under PRE (solid, black)- and POST (open, green)-infusion conditions. $*P < 0.05$: unpaired Student's *t* test of all PRE and POST events. The plot in *D* depicts a mean ± SEM representation of the PRE and POST peak amplitudes in individual cells. $*P < 0.05$: paired Student's *t* test. (*E* and *F*) Frequency (*E*) and peak frequency (*F*) of SEPs recorded before (PRE) and after (POST) infusion of $InsP_3$ into a distal astrocyte. *P* values are for a paired Mann–Whitney test. (*G–I*) Normalized cumulative histograms of amplitude (*G*), 20–80% rise time (*H*), and FWHM (*I*) for SEPs recorded before (PRE) and after (POST) infusion of 10 μM $InsP_3$ into a distal astrocyte. *P* values are for Student's *t* test. (*J–O*) Amplitudes (*J*), peak amplitudes (*K*), frequencies (*L*), peak frequencies (*M*), 20–80% rise times (*N*), and FWHM (*O*) for SEPs recorded after $InsP_3$ infusion (POST) into proximal (~25 μm, solid circles; data from Fig. 4) or distal (~240 μm, open circles) astrocytes. Boxes in *J*, *N*, and *O* represent population mean values, and *P* values there are for Student's *t* test, whereas boxes in *K–M* represent population medians, and *P* values there are for Wilcoxon's rank sum test.

receptor (independently or together) yielded significant reductions in the iiSEP frequency (Fig. 7*H*). Together, and in conjunction with previous results (7, 27, 29), these observations provide direct functional evidence that GluN2BR and GluN2DR predominantly mediate perisomatic and dendritic SEPs, respectively.

Computational Analyses Predicted an Increase in Extrasynaptic NMDAR Density with Increasing Distance from the Soma. What distribution of NMDARs would result in the emergence of an observed intraneuronal map of SEP amplitudes (Fig. 1) along the somato-apical trunk in the CA1 pyramidal neurons (12)? To address this and to quantitatively assess the role of active dendrites in regulating SEPs, we constructed a morphologically and biophysically realistic multicompartmental model of CA1 pyramidal neurons with various active and passive parameters tuned to replicate experimentally observed measurements along the somato-apical trunk (Fig. 8 *A–C* and Fig. S10 *A–C*). We simulated SEPs by incorporation and activation of biophysically realistic models of GluN2B and GluN2D receptors with their distributions set (Fig. 8*B*) according to previous observations (29). Upon performing simulations in models where receptors were distributed with different somatodendritic gradients, we found that an increase in GluN2DR density with increasing distance from the soma was essential to match the experimentally observed increase in the dendritic SEP amplitudes (Fig. 8*E*; compare Fig. 1 and Fig. S1*E*). Importantly, providing quantitative credence to our earlier observations

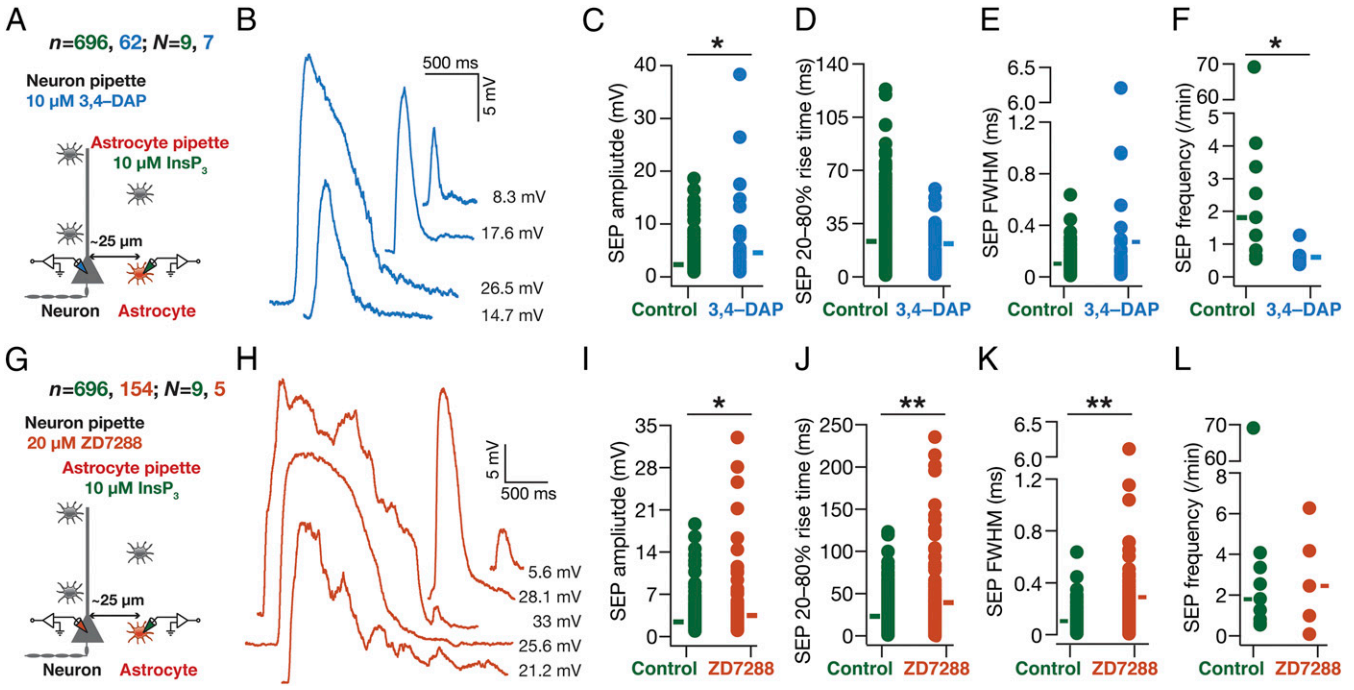

Fig. 6. In paired astrocyte–neuron recordings, *A*-type K^+ and HCN channels regulated neuronal SEPs induced by infusion of $InsP_3$ in astrocytes. (*A*) Experimental design to record iiSEPs when KA channels were blocked with 20 μM 3,4–DAP in the neuronal recording pipette. (*B*) Representative iiSEPs after blocking KA channels. (*C–F*) Amplitude (*C*), 20–80% rise time (*D*), FWHM (*E*), and frequency (*F*) of iiSEPs recorded with (3,4–DAP) and without (Control) KA-channel blockade. (*G–L*) Same as (*A–F*), but with 20 μM ZD7288 in the neuronal pipette to block HCN channels. For *C–E* and *I–K* solid rectangles represent mean values whereas for *F* and *L* they represent median values. For *F*, $*P < 0.05$, Mann–Whitney test; for *C* and *I–K*, $*P < 0.05$, $**P < 0.005$, Student's *t* test (Figs. S7 and S8).

(Figs. 2, 3, and 6), removal of KA or HCN conductances from the model resulted in similar impacts on SEP amplitude (Fig. 8*E*) and frequency (Fig. 8*F*), when identical receptors were activated across the somatodendritic structure.

Computational Analyses Demonstrated a Critical Role for Dendritic Ion Channels in Compartmentalizing the Spread of SEPs. As the intrinsic (Fig. S10) and SEP properties (Fig. 8 *D–F*) were matched in the model, we used our computational model to quantify the spatial spread of individual SEPs in the presence and absence of HCN and KA conductances. First, consistent with lower passive attenuation of low-frequency signals (22), we found that SEPs were not significantly attenuated in a passive dendritic structure (Fig. 8*G*). This lack of attenuation in the passive structure was consistent across all somatodendritic locations and was inferred from the 394-μm average SEP spread for the passive structure (Fig. 8*H*), which is close to the maximum spread of 425 μm achievable by unattenuated SEPs. However, introduction of either HCN or KA or both channels into the dendritic tree (with distributions specified in Fig. 8*C*) resulted in significant reductions in the spatial spread of SEPs. These results provide further quantitative evidence that the observed compartmentalization in SEP spread (Fig. 1) was not due to passive attenuation, but was consequent to an active role for dendritically expressed conductances.

Finally, all our experiments required delineating SEPs from synaptic potentials through blockade of synaptic transmission with TTX, which also implied the absence of somatodendritic sodium channels. As experiments without TTX would imply ambiguous results that involve synaptic potentials as well, we used our computational model to assess the role of spike-generating fast sodium (NaF) channels in the regulation of SEPs. Specifically, we incorporated NaF conductances uniformly across the somatodendritic arbor (30) and activated identical receptors across the somatodendritic structure to record the resultant SEPs across the somatoapical trunk. Expectedly, the presence of NaF channels in the somatodendritic arbor increased the SEP amplitude across the trunk, with large-amplitude SEPs resulting in dendritically initiated spikes that rarely propagated to the soma toward the generation of a somatic action potential (Fig. 8*I* and Fig. S10 *E* and *F*). Additionally, incorporation of *T*-type calcium (CaT) channels into the model, with its density increasing with distance from the soma (30), specifically enhanced SEP amplitude at distal locations compared with their proximal counterparts (Fig. S10*F*), when receptor distribution was maintained identical to the baseline scenario. Further, incorporation of both NaF and CaT conductances (with their respective somatodendritic distributions) synergistically increased the SEP amplitudes beyond values recorded with their individual presence (Fig. S10*F*) and resulted in more dendritically initiated spikes, with more numbers of them (compared with the absence of CaT channels; Fig. 8*I*) propagating to generate a somatic action potential (Fig. 8*J*).

Discussion

In this study, we unveil an important role for active dendrites in regulating the impact of gliotransmission on neurons and demonstrate that dendritically expressed ion channels actively regulate the amplitude, kinetics, frequency, and spread of slow excitatory potentials resulting from gliotransmission. We arrived at these conclusions through systematic and direct mapping of somatodendritic voltage responses to gliotransmission and through the use of pharmacological agents that were specifically applied only to the neuron under consideration. Whereas this ensured that channels in other cells in the network were not affected, our paired recording technique with specific infusion of $InsP_3$ into astrocytes provided clear lines of evidence that SEPs were consequent to gliotransmission. Importantly, our experiments involving $InsP_3$ infusion into proximal vs. distal astrocytes established a vital link between the location of an activated astrocyte and the point of origin of the associated SEP along the neuronal somatodendritic axis. In conjunction with our somatodendritic and dual recordings of spontaneous SEPs, these results provide direct functional evidence

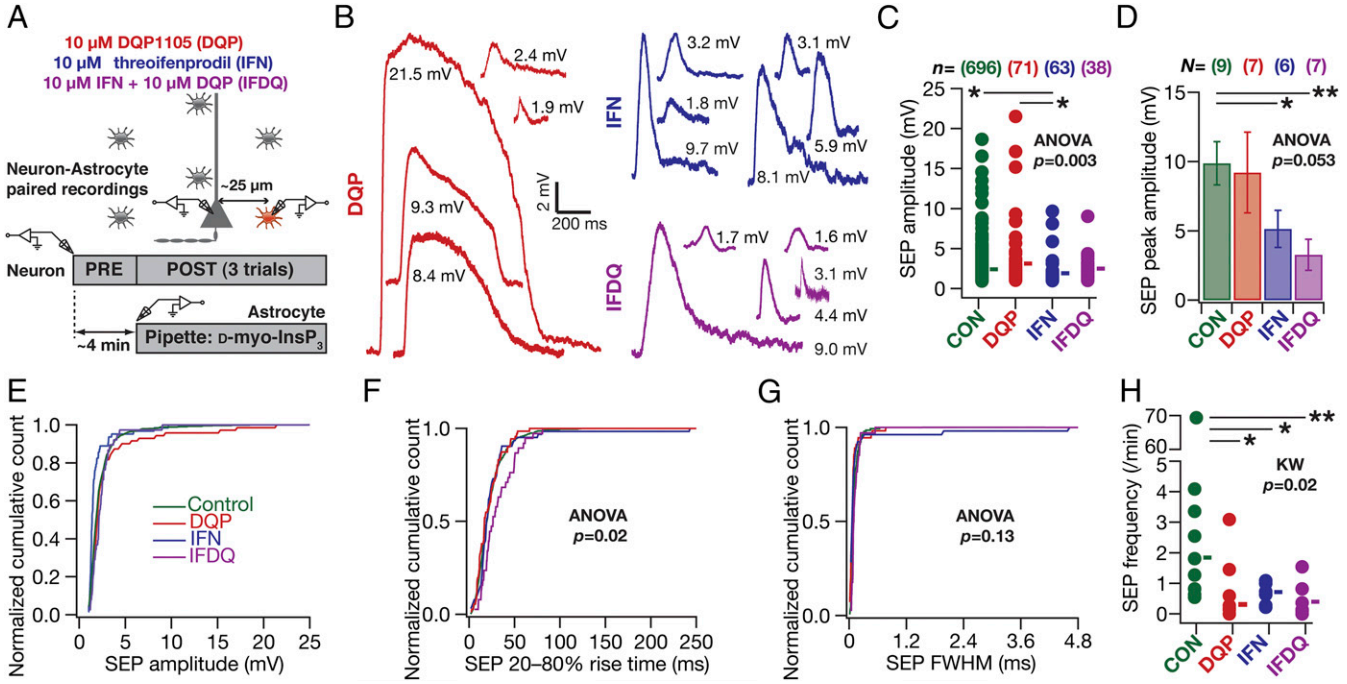

Fig. 7. In paired astrocyte–neuron recordings, SEPs were mediated by GluN2B- and GluN2D-containing NMDA receptors. (*A*) Schematic of experimental design to assess the role of different NMDAR subunits on neuronal SEPs induced by infusion of $InsP_3$ into a nearby astrocyte. The different pharmacological agents added to the artificial cerebral spinal fluid (ACSF), their (nonsaturating) concentrations, and color codes are also listed. (*B*) Representative iiSEPs recorded in the presence of blockers of different NMDAR subunits. (*C* and *D*) Amplitude (*C*) and peak amplitude (*D*) of iiSEPs recorded in the presence of blockers of different NMDAR subunits. Solid rectangles in *C* represent mean values. $*P < 0.05$, $**P < 0.005$, Student's *t* test. (*E*–*G*) Normalized cumulative histogram of iiSEP amplitude (*E*), 20–80% SEP rise time (*F*), and FWHM (*G*) recorded after infusion of $InsP_3$ into a nearby astrocyte, for experiments where specific NMDARs were blocked. For *F* the rise times for IFDQ were significantly different from Control ($P = 0.006$) and DQP ($P = 0.005$) groups with Student's *t* test. (*H*) Frequency of iiSEPs recorded in the presence of blockers of different NMDAR subunits. Solid rectangles are median frequency values. KW, Kruskal–Wallis test. $*P < 0.05$, $**P < 0.005$, Mann–Whitney test. There were no iiSEPs in two astrocyte–neuron pairs and one each in the DQP and IFDQ groups (Fig. S9). DQP, 10 μM DQP1105; IFN, 10 μM threo ifenprodil; IFDQ, 10 μM threo ifenprodil + 10 μM DQP1105.

for the dendritic origin of SEPs. Furthermore, our results unveil a functional role for the slower GluN2D-containing NMDARs in mediating the impact of gliotransmission on neurons. Specifically, using paired neuron–astrocyte recordings, we provide direct functional evidence, consistent with previous immunolocalization studies, that GluN2B- and GluN2D-containing NMDARs predominantly mediate SEPs in the perisomatic and distal dendritic locations. Using computational simulations to provide a quantitative corroboration of our experimental observations, we demonstrated that dendritic ion channels actively mediated spatiotemporal compartmentalization of SEPs. Finally, based on these simulations, we argued that SEPs could result in dendritic spikes and offered a testable prediction on a distance-dependent increase in extrasynaptic NMDARs.

Implications for Active Dendritic Regulation of the Impact of Gliotransmission. Our results provide experimental and quantitative computational evidence pointing to a critical role for active dendrites and their plasticity in structured compartmentalization of SEPs, whereby their spatiotemporal influence is confined to specific dendritic compartments (closer to the point of their origin) without necessarily propagating to distal compartments (including the soma). Such compartmentalization confers upon neurons the ability to distinguish between the activations of different astrocytes, thereby lending specificity to and enhancing the information processing capacity of neuron–astrocyte interactions. Additionally, spatially restricted events of such large amplitudes mediated by NMDARs imply a significant calcium influx into specific compartments, which could translate to localized plasticity in neuronal channels and receptors through downstream signaling components (13, 18, 31).

From a broader perspective, our demonstration of an active role for dendritically expressed ion channels in regulating the impact of gliotransmission calls for a marked rethink of the complexities associated with neuron–glia communication. Specifically, the numbers associated with dendritic 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. This additional layer of complexity introduced by the active role of dendritic ion channels in regulating the dynamics of neuron–glia signaling implies a manyfold increase in the complexity of neuronal and network computation of afferent information. Given this enhanced complexity, and given that neuron–glia interactions and any consequent plasticity would be localized to specific segments of the dendritic arbor, it is essential that future studies use direct dendritic recordings in studying neuron–glia signaling rather than focusing only on somatic physiology. Although our focus for this study has been limited to hippocampal pyramidal neurons and to two specific ion channels, the implications of our conclusions extend to other brain regions and dendritically expressed ion channels there. Given the wide diversity of expression profiles and properties of ion channels, future studies could explore the specific roles of active dendrites in regulating neuron–glia interactions in different brain regions.

Finally, and importantly, the amplitude and kinetics of several SEPs strikingly resemble those of plateau potentials (large-amplitude, long-duration depolarizations) recorded in these neurons under in vivo and in vitro conditions (31, 32). Based on this resemblance, we postulate that astrocytically originating SEPs form a substrate for the spontaneous long-duration (hundreds of milliseconds) large-amplitude plateau potentials that have been implicated in the

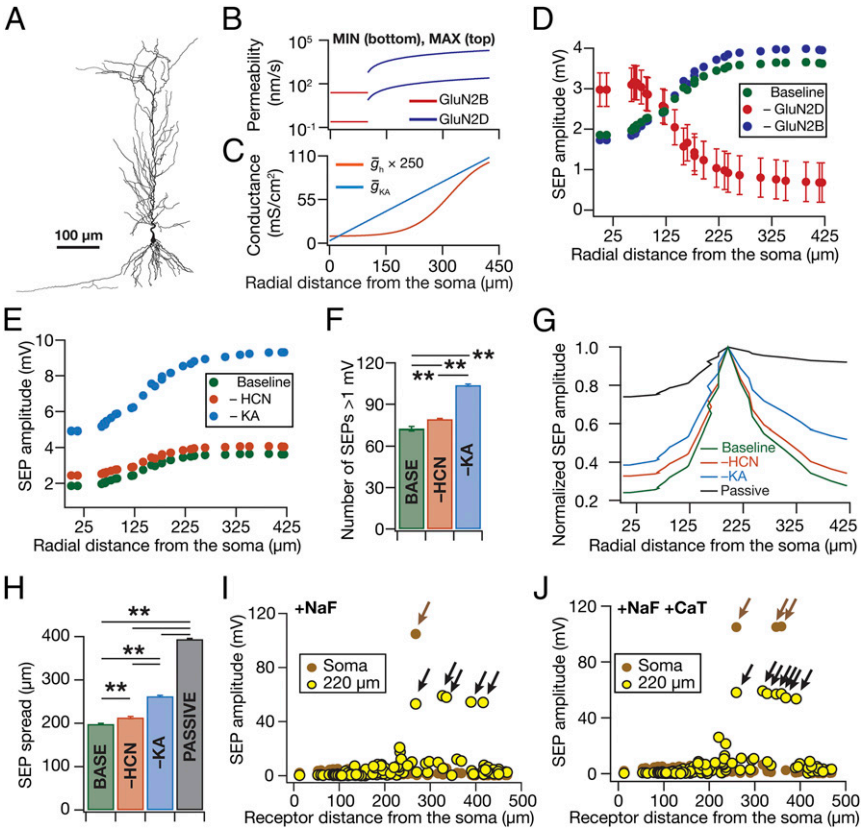

Fig. 8. Computational simulations replicated experimental findings when a somatodendritic gradient in NMDAR density was introduced and demonstrated a critical role for active dendrites in compartmentalizing the impact of gliotransmission on neurons. (*A*–*C*) Neuronal morphology (*A*) and associated somatodenritic gradients in receptor permeability (*B*) and channel conductances (*C*) used in the computational model. Receptor permeability for a given location, for any of the several trials, was chosen randomly from a uniform distribution that spanned the minimum and maximum values represented in this plot (*C*). (*D*) Simulated SEP amplitudes in the absence of GluN2B (–GluN2B) and GluN2D (–GluN2D) receptors are plotted along with those in the presence of both receptors (Baseline). (*E*) Simulated SEP amplitudes in the absence of HCN (–HCN) and KA (–KA) channels are plotted along with those in the presence of both channels (Baseline). (*F*) Number of simulated SEPs (at ∼260 μm from the soma) with amplitude >1 mV for experiments in *E*. (*G*) Normalized SEP amplitudes recorded along the somatoapical trunk, with different channel combinations (Passive implies the absence of both HCN and KA channels), when the SEP was elicited at a distance of 192 μm from the soma. (*H*) Area under the curve (AUC) for the SEP amplitudes recorded along the somatoapical trunk, under various conditions depicted in the graph, when all of the 119 receptor locations were individually activated to get SEPs across the trunk. $^{**}P < 0.005$, paired Student's *t* test. (*I* and *J*) Somatic (brown) and corresponding dendritic (∼220 μm from the soma, yellow) SEP amplitudes recorded when fast sodium channels (NaF) were incorporated into the model without (*I*) or with (*J*) *T*-type calcium (CaT) channels. Black arrows depict localized dendritic spikes and the brown arrow depicts a somatic action potential consequent to successful propagation of the corresponding dendritic spike (Fig. S10).

formation of place cells through localized plasticity (32). Specifically, behavioral regimes that lead to synchronous excitation of CA1 microcircuits could induce the release of gliotransmitters, which, through the activation of extrasynaptic NMDARs, could result in the emergence of spatiotemporally localized plateau potentials resulting in localized neuronal plasticity. As processes of single astrocytes contact 300–600 neuronal dendrites overseeing tens of thousands of synapses (2), plateau potentials mediated by gliotransmission form an ideal substrate for their postulated role in binding of information streams (33). Together, we postulate that dendritic SEPs of astrocytic origin constitute a putative cellular mechanism that can integrate network activity with biophysical and biochemical signal processing in a single neuron, thereby playing critical roles in neural coding and homeostasis.

Materials and Methods

Detailed descriptions of the surgical, electrophysiological, data analysis, and computational procedures used in this study are provided in *SI Materials and Methods*. Briefly, all surgical and electrophysiological procedures were similar to established protocols (15, 34, 35) and were performed in strict compliance with the protocols cleared by the Institute Animal Ethics Committee of the Indian Institute of Science, Bangalore, India. All neuronal and astrocytic recordings were performed under current-clamp configuration at physiological temperatures (33–35 °C) in 350-μm middle near-horizontal hippocampal slices (Bregma, –6.5 mm to –5.1 mm) from 5- to 11-wk-old male Sprague–Dawley rats. All SEPs were recorded at –70 mV. To characterize SEPs, only the events with ≥1 mV were considered for the analysis. Peak SEP amplitude (e.g., Fig. 4*D*) refers to the ensemble maximum among all SEPs recorded from the same neuron, during a given period of recording (e.g., PRE vs. POST period in Fig. 4*D*). Statistical analyses were performed using the R computing package (36) and are presented in Dataset S1 (36). Computational modeling procedures were similar to procedures in ref. 26 and involved a biophysically realistic conductance-based multicompartmental model that was constructed within the NEURON simulation environment (37), using a morphological reconstruction of a CA1 pyramidal neuron (*n123*) taken from the Neuromorpho.org database (38, 39). Passive and active (five different ion channels: Na, KDR, KA, CaT, and HCN) properties of the model neuron were set to match several somatodendritic measurements from CA1 pyramidal neurons (15–17, 26, 30, 40–42). SEPs were modeled by incorporation and activation of GluN2D- and GluN2B-containing NMDA receptors (29, 43–46), with receptor location spanning the somatoapical dendritic tree.

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