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Active dendrites support efficient initiation of dendritic spikes in hippocampal CA3 pyramidal neurons

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Abstract

CA3 pyramidal neurons are important for memory formation and pattern completion in the hippocampal network. It is generally thought that proximal synapses from the mossy fibers activate these neurons most efficiently, whereas distal inputs from the perforant path have a weaker modulatory influence. We used confocally targeted patch-clamp recording from dendrites and axons to map the activation of rat CA3 pyramidal neurons at the subcellular level. Our results reveal two distinct dendritic domains. In the proximal domain, action potentials initiated in the axon backpropagate actively with large amplitude and fast time course. In the distal domain, Na⁺ channel-mediated dendritic spikes are efficiently initiated by waveforms mimicking synaptic events. CA3 pyramidal neuron dendrites showed a high Na⁺-to-K⁺ conductance density ratio, providing ideal conditions for active backpropagation and dendritic spike initiation. Dendritic spikes may enhance the computational power of CA3 pyramidal neurons in the hippocampal network.

CA3 pyramidal neurons in the hippocampal network are critical for spatial information processing and memory¹⁻⁵. These neurons receive three different glutamatergic inputs. Proximal mossy fiber synapses activate CA3 cells efficiently, acting as ‘conditional detonators’^{6,7}. Commissural/associational synapses between CA3 cells are thought to store memories by spike timing-dependent plasticity, but whether backpropagated action potentials efficiently invade the postsynaptic dendrites in CA3 pyramidal neurons is unclear⁸⁻¹⁰. Distal perforant path synapses from the entorhinal cortex may relay information about context², but how synaptic signals are conducted to the soma via the long dendritic cable has not been resolved. Recent results have suggested that most entorhinal cortex layer 2 pyramidal neurons are grid cells¹¹, indicating that perforant path inputs may signal precise spatiotemporal information. How this information is processed by CA3 pyramidal cell dendrites remains unclear.

To understand both the induction rules of synaptic plasticity and the efficacy of distal inputs in CA3 pyramidal neurons, knowledge about the properties of the dendrites of these neurons is essential. Highly detailed information is available about the dendrites of layer 5 pyramidal cells in the neocortex and CA1 pyramidal neurons in the hippocampus¹²⁻¹⁵ (reviewed by ref. 16). In contrast, both the difficulty of maintaining CA3 pyramidal cells in *in vitro* slice preparations and the small caliber of the dendritic processes of these cells have prevented a detailed analysis by direct recordings. Recent experiments using glutamate uncaging have

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suggested that both fast Na^+ and slow NMDA spikes occur in basal dendrites of CA3 pyramidal cells, although the exact dendritic mechanisms remain unclear (J.K. Makara & J.C. Magee, *Soc. Neurosci. Abstr.*, 452.13, 2011). We studied CA3 pyramidal neurons with confocally guided subcellular patch-clamp recording techniques¹⁷⁻²⁰, which allowed us to directly examine dendritic voltage signals with microsecond temporal resolution. Our results indicate that the active properties of CA3 pyramidal neuron dendrites define two distinct dendritic domains, a proximal domain, in which action potentials efficiently backpropagate, and a distal domain, in which dendritic spikes are initiated with a low threshold.

RESULTS

We examined CA3 pyramidal neurons with improved subcellular patch-clamp recording techniques¹⁷⁻²⁰ (Fig. 1). Dendritic recordings were made at a distance of up to 403 μm from the soma¹⁸⁻²⁰ (23 recordings in stratum oriens, 37 in stratum lucidum, 134 in stratum radiatum and 4 in stratum lacunosum-moleculare; Fig. 1a-c and Supplementary Table 1). Axonal measurements were obtained up to 151 μm from the soma²¹ (Fig. 1d-f).

Action potential initiation and backpropagation

We first used subcellular recording to determine the site of action potential initiation and the rules of propagation (Fig. 2). To achieve this, we performed simultaneous somatodendritic and axosomatic recordings. Rheobase current pulses (that is, long current pulses near threshold) applied at the soma initiated low-frequency trains of action potentials in CA3 pyramidal neurons (Fig. 2a); on average, the current threshold was 192.9 ± 8.2 pA. Stimuli of higher intensity evoked action potential trains with a maximal frequency of 10.7 ± 0.3 Hz ($n = 151$), which is characteristic of CA3 pyramidal cells²².

Using simultaneous somatodendritic recording, we first detected the action potential evoked by somatic current pulses at the somatic recording site and then at the dendritic recording site (Fig. 2a). In contrast, simultaneous axosomatic recording revealed that the action potential in the proximal axon preceded the somatic waveform (Fig. 2a). This temporal sequence was highly consistent from trial to trial and among cells, suggesting that robust axonal action potential initiation was followed by dendritic backpropagation¹⁶.

We next examined the distance dependence of peak amplitude and duration of the dendritic action potential in CA3 pyramidal neurons (Fig. 2b,c). Notably, the peak action potential amplitude declined sigmoidally as a function of distance from the soma, with little change occurring in the first 100 μm ($n = 43$ somatodendritic recordings; Fig. 2b and Supplementary Table 1). On average, the amplitude of the action potential at the dendrite between 50 and 100 μm was $91 \pm 2\%$ of that at the soma. Furthermore, the action potential half-duration increased only minimally as a function of distance, again with virtually no changes occurring in the first 100 μm (Fig. 2c). On average, the half-duration of the action potential at the dendrite between 50 and 100 μm was $106 \pm 2\%$ of that at the soma (11 somatodendritic recordings). These results indicate that action potentials propagate into the proximal dendrites of CA3 pyramidal neurons with large amplitude and fast time course.

To further determine the exact site of action potential initiation, we plotted action potential latency and maximal rate of rise ($(dV/dt)_{\text{max}}$) against distance (Fig. 2d,e). Analysis of the dataset comprised of 43 somatodendritic and 11 axosomatic recordings revealed that the shortest latency was observed in the axon 75 μm from the center of the soma^{23,24}. These results indicate that the primary site of action potential initiation under these conditions is the proximal part of the axon. Consistent with this idea, the maximal rate of rise of the action potential was highest at proximal axonal sites, suggesting a maximal Na^+ conductance in this region. In conclusion, these results suggest that during application of

somatic rheobase stimuli the action potential is initiated in the proximal axon and subsequently propagated back into the dendrites. Similar results were obtained at $\sim 22^\circ\text{C}$ and at near-physiological temperature ($\sim 33^\circ\text{C}$; Supplementary Fig. 1).

CA3 pyramidal neurons fire bursts of action potentials (typically 3–5) under a variety of conditions both *in vitro* and *in vivo*²⁵. To examine whether action potential backpropagation was activity dependent, we repetitively stimulated CA3 pyramidal neurons with trains of brief somatic current pulses (Fig. 3). Trains of pulses were applied at frequencies of 20, 50 and 100 Hz (Fig. 3a–c) and the properties of the fifth action potential were compared with those of the first action potential for both somatic and dendritic recording sites. Action potentials propagated efficiently into the dendrites even during high-frequency trains. The ratio of action potential amplitude of the fifth action potential over that of the first action potential was in the range of ~ 0.9 – 0.5 (Fig. 3d). Similarly, the ratio of action potential half-duration of the fifth action potential over that of the first action potential was in the range of ~ 1.0 – 1.5 (Fig. 3e). These results indicate that backpropagation of action potentials into the dendrites during repetitive activity is highly reliable over a wide range of frequencies.

To distinguish whether efficient action potential backpropagation in CA3 pyramidal neurons is caused by cable properties of the neurons²⁶ or by active conductances, we applied previously recorded somatic action potential waveforms as voltage-clamp commands to the soma. The resulting dendritic voltage changes under these conditions in the presence of $0.5\ \mu\text{M}$ tetrodotoxin (TTX) were compared with naturally backpropagating action potentials (Supplementary Fig. 2). Dendritic peak amplitudes of passively propagated action potentials were significantly smaller than those of naturally propagated action potentials in the same cells, indicating that voltage-gated Na^+ channels provide a substantial contribution to active dendritic action potential propagation (seven somatodendritic recordings, $P < 0.05$; Supplementary Fig. 2a,b,d). In contrast, control experiments with two pipettes both located at the soma yielded voltage signals with similar amplitude (five dual somatic recordings, $P > 0.2$), confirming the validity of the experimental approach (Supplementary Fig. 2c).

Dendritic channel distribution

To determine the ionic mechanisms underlying the efficient backpropagation of action potentials into the dendrites, we mapped the density of dendritic voltage-gated Na^+ and K^+ conductance over the entire somatodendritic domain of CA3 pyramidal cells (Fig. 4). Under conditions in which voltage-gated Na^+ currents were pharmacologically isolated (that is, with Cs^+ -rich intracellular solution in the pipette), transient Na^+ currents were recorded in both somatic and dendritic outside-out patches (Fig. 4a). These currents were blocked by $0.5\ \mu\text{M}$ extracellular TTX, indicating that they are mediated by voltage-gated Na^+ channels. Under conditions in which voltage-gated K^+ currents were isolated (that is, with a K^+ -rich intracellular solution and test pulses to $+70\ \text{mV}$, close to the reversal potential of the Na^+ current), K^+ currents containing inactivating A-type and delayed rectifier K^+ components were found in both somatic and dendritic membrane patches (Fig. 4b,c). To separate the components, we applied a pulse protocol with two different prepulses ($-120\ \text{mV}$ and $-40\ \text{mV}$). An inactivating, A-type current component, which was isolated by subtraction of responses with -40-mV prepulses from those with -120-mV prepulses, was blocked by $5\ \text{mM}$ extracellular 4-aminopyridine (4-AP; Fig. 4b), but not by $20\ \text{mM}$ tetraethylammonium (TEA, data not shown). In contrast, a delayed rectifier current component, which was isolated using depolarizing prepulses, was largely suppressed by $20\ \text{mM}$ extracellular TEA (Fig. 4c).

To analyze the spatial distribution of Na^+ , A-type K^+ and delayed rectifier K^+ current components, we plotted current density against distance from the soma (Fig. 4d–f). Notably, the density of the different components showed differential distance dependence. For the

Na⁺ current, the apparent density decreased from the soma to the proximal dendrites and then increased from the proximal to the distal dendrites (Spearman rank correlation analysis, $n = 41$ dendritic outside-out patches, $P < 0.01$; Fig. 4d). In contrast, the dendritic A-type K⁺ current density increased continuously from the soma to the distal dendritic region ($n = 38$ dendritic outside-out patches, $P < 0.01$; Fig. 4e). Finally, the delayed rectifier K⁺ current density was not significantly dependent on distance ($P > 0.1$; Fig. 4f). Conversion of dendritic current density into conductance density revealed that the average ratio of Na⁺ to total K⁺ conductance density was 0.72 (Supplementary Table 2). Thus, CA3 pyramidal neuron dendrites show a high Na⁺-to-K⁺ current ratio in comparison with other types of neurons^{12,20}. Comparable results were obtained with cell-attached patches (Supplementary Fig. 3).

To examine whether dendritic channels differ from somatic channels in the voltage dependence of gating, we measured activation and inactivation curves for both dendritic and somatic outside-out patches (Supplementary Fig. 4). The Na⁺ channel ($P < 0.01$) and A-type K⁺ channel ($P < 0.05$) activation had more negative midpoint potential in the dendrite than in the soma, whereas activation curves of delayed rectifier K⁺ channels were not significantly different ($P > 0.5$; Supplementary Table 3).

Dendritic spikes in CA3 pyramidal neurons

The high dendritic Na⁺ channel density and the more negative activation threshold of dendritic Na⁺ channels raise the possibility that CA3 pyramidal neurons may generate dendritic spikes²⁷⁻³¹. Indeed, short current pulses applied to the dendrite evoked dendritic spikes with high efficiency (Fig. 5). Dendritic spikes occurred in complete isolation with stimuli slightly above threshold, but were coupled to subsequent axosomatic spikes with larger stimuli (Fig. 5a). Similarly, multi-exponential current and conductance waveforms injected into the dendrite robustly initiated dendritic spikes, which were recognized as a positive deviation from the exponential time course of the voltage waveform (Fig. 5b and Supplementary Fig. 5). Analysis of voltage-current relations revealed that dendritic spikes were all-or-none events and often occurred in isolation from axosomatic spikes (Fig. 5c). The probability of dendritic spike initiation in CA3 pyramidal cells was high, reaching a value close to 1 for distances $>100 \mu\text{m}$ ($P < 0.001$; Fig. 5d).

It is generally thought that distal synapses are much less efficient at activating CA3 pyramidal neurons than proximal synapses as a result of filtering of synaptic potentials along the dendritic cable^{32,33}. We therefore analyzed the distance dependence of axosomatic and dendritic spike threshold (Fig. 5e,f). For axosomatic spikes, the current threshold increased significantly as a function of distance, as expected from cable theory ($P < 0.05$; Fig. 5e). For dendritic spikes, however, the threshold decreased substantially with distance ($P < 0.001$; Fig. 5f), suggesting that a small number of unitary inputs may be sufficient for initiation. Similar results were obtained with multi-exponential conductance waveforms applied in the dynamic-clamp configuration (Supplementary Fig. 5). The two threshold curves crossed at a distance of $\sim 125 \mu\text{m}$ (Fig. 5e,f). Thus, CA3 pyramidal neurons had two distinct functional dendritic domains. In the proximal domain, action potentials backpropagate with nearly constant amplitude and time course, and excitatory postsynaptic currents (EPSCs) trigger somatic spikes. In contrast, in the distal domain, EPSCs initiate dendritic spikes with a low threshold. Thus, the activity of a small number of converging inputs may be sufficient to trigger dendritic spikes in CA3 pyramidal neurons.

Ionic mechanisms and functional effect

To identify the ion channels underlying dendritic spikes, we tested the effects of blockers of voltage-gated Na⁺ and Ca²⁺ channels (Fig. 6). Global application of $0.5 \mu\text{M}$ TTX in the bath

blocked both axosomatic and dendritic spikes (3 of 3 cells; Fig. 6a, b). In contrast, local application of 1 μM TTX to the dendrite near the recording site selectively blocked the dendritic spike, but left the axosomatic spike unaffected (4 of 4 cells; Fig. 6c,d). This suggests that the Na^+ channels that are responsible for the dendritic spike are located at the dendrite, and further confirms the absence of spillover of TTX from the dendrite to the perisomatic compartment. Finally, application of 200 μM Cd^{2+} to the bath did not affect the dendritic spike amplitude, kinetics or threshold (6 of 6 cells; Fig. 6e,f). Taken together, these results indicate that dendritic spikes are mediated by voltage-gated Na^+ channels, rather than by Ca^{2+} channels.

To determine the effect of dendritic spikes on axosomatic output of CA3 pyramidal neurons, we examined the effect of dendritic spikes evoked by synaptic event-like stimuli on the somatic voltage (Fig. 7a-c). Dendritic spikes attenuated substantially during propagation from the dendrites to the soma (Fig. 7d). However, despite attenuation, they had a substantial effect on the output of CA3 pyramidal neurons. Initiation of dendritic spikes was correlated with an acceleration and an overshoot in the rising phase of the somatic excitatory postsynaptic potential (EPSP; Fig. 7b,e), suggesting that dendritic spikes could enhance the efficacy and speed of axosomatic spike generation. Consistent with this hypothesis, local application of TTX to the dendrite blocked dendritic spikes and increased the threshold for the generation of axosomatic spikes in parallel (Fig. 7c,f). Comparing our results in CA3 pyramidal neurons with previous findings on CA1 pyramidal neurons^{13,14,27,30} suggests that dendritic properties differ between the two types of neurons. To examine these differences directly under identical conditions, we performed similar experiments in CA1 pyramidal neuron dendrites (Supplementary Figs. 6 and 7).

We found that, in CA1 pyramidal neurons, action potential backpropagation in the proximal dendrite showed markedly larger attenuation, broadening and activity dependence (Supplementary Fig. 6), dendritic Na^+ channel density was lower and more uniform (Supplementary Fig. 7b,c), and dendritic spikes were evoked with lower probability (Supplementary Fig. 7d,e). Thus, hippocampal pyramidal neurons in different subfields (CA3 versus CA1) differ in action potential propagation, ion channel distribution and local signal processing in their dendrites.

DISCUSSION

Our results represent, to the best of our knowledge, the first analysis of dendritic function of CA3 pyramidal neurons by subcellular patch-clamp recording. Together with previous results^{13,34}, our data suggest that different types of principal cells in the trisynaptic hippocampal circuit differ substantially in their dendritic properties (Supplementary Table 4). Whether our conclusions also hold for very fine distal dendrites (including apical tuft, apical oblique and distal basal dendrites) remains to be determined³⁵.

The different dendritic properties of CA3 pyramidal cell dendrites confer specific action potential backpropagation rules. In CA3 pyramidal neurons, there is a region of 100 μm from the soma (approximately corresponding to the stratum lucidum) in which action potential backpropagation is only moderately decremental and is associated with minimal changes in time course. In contrast, in CA1 pyramidal neurons, action potential amplitude declined exponentially and action potential duration increased as a function of distance¹³ (Supplementary Fig. 6). Furthermore, action potential backpropagation in CA3 cells is reliable for stimulation frequencies up to 100 Hz, whereas it is more activity-dependent in CA1 pyramidal neurons¹³. Thus, in CA3 neurons, backpropagated action potentials provide large and temporally precise feedback signals, which are ideally suited for the induction of

spike timing-dependent plasticity in commissural/associational synapses over a wide range of activity patterns⁸⁻¹⁰.

The specific dendritic properties also lead to unique characteristics of local electrogenesis. In CA3 neurons, dendritic spikes were observed with a probability of near 1 at a distance of $>100\ \mu\text{m}$ from the soma, typically occurred separately from axosomatic spikes, and showed a low current and conductance threshold at distal dendritic locations. In contrast, in CA1 pyramidal neurons, dendritic spikes were observed in a smaller fraction of neurons, were frequently coupled to axosomatic spikes and showed a high threshold^{27,30} (Supplementary Fig. 7). Several factors may explain the high probability of dendritic spike initiation in CA3 pyramidal cells: the passive cable properties, particularly the increase in input impedance as a function of distance^{26,36}, the high Na^+ -to- K^+ conductance ratio and the increase of Na^+ conductance density as a function of distance, and the more negative midpoint potential of the Na^+ channel activation curve. This hypothesis has been corroborated by simulations using active cable models of CA3 pyramidal cells with a high Na^+ -to- K^+ conductance ratio, which can reproduce both the moderate attenuation of backpropagating action potentials and the low threshold of dendritic spike initiation (S.K. and P.J., unpublished data).

On the basis of cable theory, it is generally assumed that proximal mossy fiber synapses activate CA3 pyramidal cells more powerfully than distal synapses^{6,32,37}. However, we found that dendritic spike initiation followed an inverse synaptic efficacy rule. As the initiation threshold for dendritic spikes decreased with distance, our results suggest that dendritic spike initiation may represent a prevalent computational mode of CA3 pyramidal neurons *in vitro* and *in vivo*.

The presence of dendritic spikes enriches the computational repertoire of CA3 pyramidal neurons in multiple ways. First, dendritic spikes may boost the efficacy and speed of generation of axosomatic action potentials, especially if dendrosomatic propagation is enhanced by activation of proximal mossy fiber synapses³⁸. Second, dendritic spikes implement a mechanism for local submillisecond coincidence detection and multiplicative computations in dendrites of CA3 pyramidal cells^{39,40}. This may increase the memory capacity of single CA3 pyramidal neurons⁴⁰. Third, dendritic spikes will shape synaptic plasticity rules, relieving the Mg^{2+} block of NMDA receptors^{29,41}. As axosomatic spikes are not strictly required for this form of activity, dendritic spikes could be the basis of heterosynaptic forms of plasticity at synapses on CA3 pyramidal neurons^{42,43}.

Finally, dendritic spikes will help to process spatially and temporally precise grid cell input from the entorhinal cortex via the perforant path^{11,44,45}. Dendritic spikes may implement a coincidence detection mechanism for incoming grid cell input with different spatial phase and frequency, reducing the broad activation at multiple points in behavioral space to the selective activation at a single point. Thus, dendritic spikes may contribute to the transformation of grid cell activity in the entorhinal cortex into place cell activity in the hippocampal CA3 region.

ONLINE METHODS

Subcellular patch-clamp recording

Transverse hippocampal slices (thickness, $350\ \mu\text{m}$) were prepared from the brains of 24- to 29-day-old Wistar rats. Rats were lightly anesthetized using isoflurane (Forane, Abbott) and killed by rapid decapitation, in accordance with national and institutional guidelines. Experiments were approved by the Bundesministerium für Wissenschaft und Forschung (A. Haslinger, Vienna). Slices were cut in ice-cold sucrose-containing physiological saline using a vibratome (Leica VT1200), incubated in a maintenance chamber filled with sucrose-saline

at ~36 °C for 45 min, and subsequently stored at ~22 °C (refs. 17,46). Slices were then individually transferred into a recording chamber perfused with standard physiological saline. Recordings were performed at ~22 °C (range, 20–25 °C) or, in a subset of experiments, at near-physiological temperature (~33 °C). Recordings were preferentially obtained from area CA3b, and from area CA1 for reference purposes. Recorded neurons had their somata in stratum pyramidale; displaced cells were avoided.

To obtain recordings from dendrites and axons of CA3 pyramidal neurons, we adopted the following experimental strategy^{18–20}. First, a somatic recording configuration was obtained, using an internal solution containing Alexa Fluor 488 (100 μM, Invitrogen). Second, after ~10 min of somatic whole-cell recording, fluorescently labeled dendrites or axons were traced from the CA3 pyramidal neuron soma into the stratum lacunosum-moleculare (apical dendrites) or oriens-alveus (basal dendrites or axons) using a Nipkow spinning disk confocal microscope (Ultraview live cell imager, PerkinElmer, equipped with an Orca camera, Hamamatsu, and an argon/krypton laser; excitation wavelength of 488 nm). Exposure times were minimized to avoid phototoxicity. Finally, fluorescent and infrared differential interference contrast (IR-DIC) images were compared and CA3 pyramidal neuron dendrites or axons were patched under IR-DIC²⁰. Axonal recordings were obtained at axon blebs, artificial enlargements formed by the slicing procedure near the surface of the slice²¹.

Patch pipettes were pulled from thick-walled borosilicate glass tubing (outer diameter = 2 mm, inner diameter = 1 mm) with a horizontal pipette puller (P-97, Sutter Instruments). For somatodendritic recordings, patch pipettes had resistances of 3–7 MΩ (soma) and 8–23 MΩ (dendrite). For outside-out and cellattached patch recording, typically pipettes with resistance of 9–23 MΩ were used. For comparison of channel density between soma and dendrites (Fig. 4), pipettes had similar size and geometry. For cell-attached recordings, pipettes were coated with dental wax (Pluradent). Current- and voltage-clamp recordings were performed with a Multiclamp 700A amplifier (Molecular Devices). Series resistance was 8–80 MΩ; for quantitative measurements, only recordings with series resistance < 60 MΩ were used. Experiments in which somatic or dendritic resting potentials were more positive than –55 mV were also rejected. Pipette capacitance and series resistance compensation (bridge balance) were applied throughout current-clamp experiments. Bridge balance was checked repeatedly and readjusted as required. CA3 pyramidal neurons were held at the resting membrane potential (–65.9 ± 0.4 mV at the soma, 151 recordings were used).

Signals were low-pass filtered at 10 kHz in current-clamp recordings and 4 kHz in voltage-clamp recordings and digitized at a sampling rate of 20 kHz with a CED power 1401 interface (Cambridge Electronic Design). Pulse protocols were generated using custom-made data acquisition software (FPulse 3.33, U. Fröbe) running under Igor Pro 6.21 or 6.22 (WaveMetrics). To record voltage-gated Na⁺ current in outside-out patches, we generated a pulse sequence comprised of a 100-ms prepulse to –120 mV and a 30-ms test pulse to 0 mV. To measure voltage-gated K⁺ currents, we applied a pulse sequence consisting of a 100-ms or 200-ms prepulse to either –120 mV or –40 mV followed by a 200-ms or 250-ms test pulse to 70 mV. For inactivation curves of A-type K⁺ current, 2- to 5-s prepulses to potentials between –120 mV and –10 mV were used. In all cases, the holding potential before and after the pulse sequence was –90 mV. Voltage protocols were applied to outside-out patches once every ~10 s. Leak and capacitive currents were subtracted online using a ‘P over –8’ or ‘P over –4’ correction procedure. To assess the active components of action potential backpropagation, we recorded an action potential in the current-clamp mode and digitized and subsequently applied it as voltage-clamp command at the soma in the same cell using FPulse. In this set of experiments, correction and prediction were enabled (80–85%) and the bandwidth was 4–5 kHz. In some of the figures, residual capacitance

transients were blanked for clarity. In both experiments with EPSC-like current waveforms and dynamic-clamp experiments, a current or conductance represented by the sum of two exponentials, with a rise time constant of 0.25 ms and a decay time constant of 5 ms^{47,48}, was injected into the dendrite. For dynamic-clamp experiments, we used a system that was either based on a digital signal processor card⁴⁹ or an analog converter that calculated currents by multiplication of imposed conductances and measured voltages in real time. Reversal potential was set to 0 mV in all cases. In dynamic-clamp experiments, only dendritic recordings with series resistance ranging from 27–35 MΩ were used and series resistance compensation was monitored at short time intervals. Furthermore, recordings were restricted to the very early phase of whole-cell recording, in which the series resistance was typically lowest.

Biocytin labeling

For analysis of neuron morphology after recording, slices were fixed overnight in 2.5% paraformaldehyde (wt/vol), 1.25% glutaraldehyde (wt/vol) and 15% picric acid (wt/vol) in 100 mM phosphate buffer, pH 7.3. After fixation, slices were incubated in 1% hydrogen peroxide (wt/vol) and shockfrozen in liquid nitrogen. Subsequently, the tissue was treated with phosphate buffer containing 1% avidin–biotinylated horseradish peroxidase complex (ABC, wt/vol; Vector Laboratories) overnight at 4 °C. Excess ABC was removed by several rinses with phosphate buffer, before development with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (wt/vol) and 0.01% hydrogen peroxide. Subsequently, slices were rinsed in phosphate buffer several times and embedded in Mowiol (Höchst). CA3 pyramidal neuron dendrites were identified based on the high density of spines and the large thorny excrescences in stratum lucidum⁵⁰. Conversely, CA3 pyramidal neuron axons were unequivocally identified by the lack of spines.

Solutions and chemicals

The standard physiological external solution contained 125 mM NaCl, 25 mM NaHCO₃, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 1 mM MgCl₂ and 25 mM D-glucose (equilibrated with 95% O₂ and 5% CO₂ gas mixture). The sucrose-containing external solutions contained either 64 mM NaCl and 120 mM sucrose or 87 mM NaCl and 75 mM sucrose, with 10 mM D-glucose in both cases. TTX (0.5 or 1 μM in physiological saline or HEPES-buffered saline) was applied either via bath perfusion or by local application with a pressure application system (Picospritzer 2, General Valve). Pressure pulses had durations of 0.5 s and amplitudes of ~10 psi. Cd²⁺ was applied in the bath at a concentration of 200 μM (this concentration was saturating for Ca²⁺ channel block, but below that severely affecting A-type K⁺ channels)⁵¹. TTX was purchased from Biotrend; TEA and 4-AP were from Sigma-Aldrich.

For whole-cell recording and K⁺ current recording in outside-out patches, we used a K⁺-rich internal solution that contained 135 mM potassium gluconate and 0.1 mM EGTA, or 120 mM potassium gluconate and 10 mM EGTA, 20 mM KCl, 2 mM MgCl₂, 2 mM Na₂ATP, 0.2% biocytin (wt/vol) and 10 mM HEPES, pH adjusted to 7.3 with KOH. We added 100 μM Alexa Fluor 488 to the solution for somatic recording electrodes. In a subset of experiments, 10 mM phosphocreatine and 0.4 mM GTP were included in both somatic and dendritic pipettes to minimize rundown of voltage-gated currents. In the majority of Na⁺ current recordings in outside-out patches, the internal solution contained 120 mM cesium gluconate, 20 mM CsCl, 0.1 or 10 mM EGTA, 2 mM MgCl₂, 2 mM Na₂ATP, and 10 mM HEPES, pH adjusted to 7.3 with CsOH. For cell-attached Na⁺ current recording, the pipette solution contained 125 mM NaCl, 2 mM CaCl₂, 1 mM MgCl₂, 20 mM TEA, 3 mM 4-AP, 10 mM D-glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH. For cell-attached K⁺ current recording, the pipette solution was composed of 125 mM NaCl, 2 mM CaCl₂, 1 mM

MgCl₂, 2.5 KCl, 1 μM TTX, 25 mM D-glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH.

Data analysis

Analysis was performed using Igor Pro (Wavemetrics), C-Stimfit (C. Schmidt-Hieber, University College London, and P.J.; <http://code.google.com/p/stimfit/>), Excel (Microsoft) and Mathematica 8.01 (Wolfram Research). Action potential peak amplitude was measured from threshold (50 V s⁻¹ for soma or axon and 10–50 V s⁻¹ for dendrite). Action potential duration was determined at half-maximal amplitude, using threshold and peak as reference points. Latency differences between somatic and dendritic action potentials were quantified using the time points at half-maximal amplitude in the action potential rising phase. To determine the action potential initiation site, we fit action potential latencies with a third-order polynomial function. Average spike frequency was determined from the number of action potentials during a 1-s depolarizing current pulse. Amplitude of dendritic spikes was measured after subtraction of appropriately scaled subthreshold responses. Activation and inactivation curves were fitted by Boltzmann functions of the form $f(V) = A[1 + \exp((V - V_{0.5})/k)]^{-1} + B$, where $V_{0.5}$ is the midpoint potential, k is the slope factor, and A and B are amplitude factors. For display of activation and inactivation data, values were normalized to the maximal value of the fitted curve. Statistical significance of differences between midpoint potentials at soma and dendrites was tested by bootstrap analysis.

Values indicate mean ± s.e.m. Membrane potentials are given without correction for liquid junction potentials. Significance of differences was assessed by two-sided nonparametric Wilcoxon signed rank or Mann-Whitney tests at a significance level of $P < 0.05$. Significance of correlations was tested using the Spearman rank correlation test (Igor Pro). For the calculation of current and conductance density, membrane patch area was estimated from pipette resistance using a previously established empiric relation⁵². Distances were measured from the center of the soma to the tip of the dendritic or axonal recording pipette. Video images were acquired with DScaler (the DScaler project team) and analyzed using ImageJ (W. Rasband, US National Institutes of Health). The width of the different layers was measured using biocytin-labeled CA3 pyramidal neurons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Subcellular patch-clamp recording from dendrites and axons of CA3 pyramidal neurons. (**a-c**) Dendritic recording from CA3 pyramidal neurons. A confocal image of a CA3 pyramidal cell filled with 100 μ M Alexa Fluor 488 via the somatic recording pipette during the experiment is shown in **a**. The dendritic recording site is 218 μ m from the soma. An infrared differential interference contrast video image of the soma (top) and the apical dendrite (bottom) of the same CA3 pyramidal neuron with the patch pipettes attached is shown in **b**. A light micrograph of a CA3 cell filled with biocytin during recording and labeled using 3,3'-diaminobenzidine (DAB) as chromogen is shown in **c**. The bottom panel shows an expanded view corresponding to the dashed box shown in the top panel. Arrowheads in **c** indicate thorny excrescences, the postsynaptic spines of hippocampal mossy fiber synapses. (**d-f**) Axonal recording from CA3 pyramidal neurons. A confocal image of a CA3 pyramidal neuron filled with 100 μ M Alexa Fluor 488, showing an axon bleb at the surface of the slice, is presented in **d**. The axonal recording site is 90 μ m from the soma. A corresponding infrared differential interference contrast video image is shown in **e**. *Post hoc* biocytin labeling of the same CA3 cell using DAB as chromogen is shown in **f**. The axon was unequivocally identified by the complete lack of spines. Arrowhead in **f** indicates axon bleb. Photomicrographs in **a**, **d** and **f** represent collages of images at slightly different focal planes. Images in **a** and **b** were obtained from the same cell; images in **d-f** were obtained from another cell.

Figure 2.

Action potentials backpropagate into the dendrites of CA3 pyramidal neurons with large amplitude and fast time course. **(a)** Train of action potentials (APs) evoked by somatic current injection, simultaneously recorded at soma and dendrites (left) and soma and axon (right). Black traces represent somatic voltage and corresponding current, red traces represent dendritic voltage and current, and blue traces represent axonal voltage and current. The current intensity was 325 pA (left) and 675 pA (right). Bottom traces show first action potential in the 1-s train on an expanded timescale. The dendritic recording site is 144 μm from the soma and the axonal recording site is 54 μm from the soma. **(b,c)** Summary plot of action potential peak amplitude measured from threshold **(b)** and duration at half-maximal amplitude **(c)** in 43 somatodendritic recordings plotted against distance (positive distance, apical dendrite; negative distance, basal dendrite; zero distance, soma). Somatic rheobase current stimuli were used in all cases. Dashed curves represent a fitted Boltzmann function **(b)** and the results of linear regression **(c)** for data points from soma and apical dendrites. **(d,e)** Summary plot of action potential latency measured at half-maximal amplitude **(d)** and maximal rate of rise (dV/dt_{max} , **e**) in 43 dendritic and 11 axonal recordings plotted against distance (positive distance, axon; negative distance, dendrites). Somatic rheobase current stimuli were used in all cases. The green curve represents a third-order polynomial function fitted to the data points. The smallest latencies were measured at a distance of 75 μm from the center of the soma, representing the action potential initiation site.

Figure 3.

Dendritic action potential backpropagation in CA3 pyramidal cells shows only moderate activity dependence. **(a-c)** Action potentials evoked by trains of ten brief current pulses applied to the soma at a frequency of 20, 50 and 100 Hz. Note that action potentials were efficiently propagated even during high-frequency trains. Black represents somatic voltage and red represents dendritic voltage. The recording site on the apical dendrite is 144 μm from the soma. **(d)** Ratio of action potential amplitude for the fifth action potential over that of the first action potential in the train. Dashed lines represent the results of linear regression. **(e)** Ratio of action potential half-duration for the fifth action potential over that of the first action potential in the train. The ratio was close to 1, indicating that action potentials during repetitive activity were propagated with fast time course.

Figure 4.

High Na⁺-to-K⁺ conductance ratio and distinct conductance gradients in CA3 pyramidal neuron dendrites. **(a)** Na⁺ current (average from 20–33 single traces, respectively) evoked in outside-out patches from soma and apical dendrite (150 μm). Test pulse potential was 0 mV. Na⁺ current was recorded with Cs⁺-internal solution. Left, soma; right, dendrite; top, control; bottom, currents in the presence of 0.5 μM TTX in the bath. We used outside-out patches; leak and capacitive currents were subtracted by a ‘P over –8’ correction procedure. **(b)** A-type K⁺ current (average from 9–42 single traces) evoked in outside-out patches from soma and apical dendrite (153 μm). K⁺ current was recorded with K⁺-internal solution. A-current was isolated by subtraction of traces with a –40-mV prepulse from those with a –120-mV prepulse. Left, soma; right, dendrite; top, control; bottom, currents in the presence of 5 mM 4-AP in the bath. **(c)** Delayed rectifier K⁺ current (average from 5–10 single traces) evoked in outside-out patches from soma and apical dendrite (134 μm). Delayed rectifier K⁺ current was isolated by a –40-mV prepulse. Left, soma; right, dendrite; top, control; bottom, currents in the presence of 20 mM TEA in the bath. Pulse protocols in **b** and **c**: prepulse to –120 mV or –40 mV, test pulse to 70 mV. **(d–f)** Plot of Na⁺ current density **(d)**, A-type K⁺ current density **(e)** and delayed rectifier K⁺ current density **(f)** against distance from the soma. Dashed lines represent the results of linear regression of data points from apical dendrite. Data from 12, 12 and 12 somatic (black circles) and 41, 38 and 38 dendritic patches (red circles).

Figure 5.

Efficient initiation of dendritic Na⁺ spikes in CA3 pyramidal neurons. **(a)** Examples of dendritic spikes in CA3 pyramidal neurons. Top traces, 5-ms current pulses (intensity: 600 pA, 960 pA and 1,180 pA). Upper middle traces, subthreshold response. Lower middle traces, isolated dendritic spike. Bottom traces, dendritic spike followed by an axosomatic spike. Black traces represent somatic signals and red traces represent dendritic signals. **(b)** Dendritic spikes evoked by EPSC-like current waveforms in CA3 pyramidal neurons. Top, EPSC-like currents used as stimuli (rise time constant = 0.25 ms, decay time constant = 5 ms). The peak current was increased from 300 pA to 2,700 pA in 600-pA steps. Bottom, corresponding EPSP-like voltage waveforms. Black traces represent somatic signals and red traces represent dendritic signals. **(c)** All-or-none characteristics of dendritic spikes. Plot of peak amplitude of the dendritic signal against intensity of the current pulse. Amplitude was measured after subtraction of scaled subthreshold responses. The stepwise increase at ~700 pA corresponds to the initiation of the dendritic spike, whereas the second increase at ~1.2 nA reflects initiation and backpropagation of the axosomatic action potential (bAP). Data shown in **a** and **c** were taken from the same cell; data shown in **b** were taken from a different cell. The recording sites on apical dendrite are 133 μm (**a,c**) and 142 μm (**b**) from the soma. **(d)** Histogram of number of recordings with (gray bars) or without dendritic spikes (open bars) at different distances from the soma. Note that dendritic action potential initiation robustly occurred at distances >100 μm. Stimulation intensity = 100 pA to 3 nA. The blue curve shows the corresponding probability of dendritic spike initiation (right axis), as obtained by fitting with a Boltzmann function. **(e,f)** Plot of initiation threshold for axosomatic spikes (**e**) and dendritic spikes (**f**) as a function of distance. Data in **e** and **f** are from the same set of recordings. In distal recordings, current stimuli easily evoked dendritic spikes, but occasionally failed to trigger axosomatic spikes even at high intensity (no corresponding data points in **e**). Open circles indicate threshold values for 5-ms current pulses. Filled circles indicate threshold values for EPSC-like current waveforms.

Figure 6.

Dendritic spikes are mediated by voltage-gated Na⁺ channels. **(a)** Schematic illustration of the current-clamp recording configuration (CC), combined with bath and local application of TTX. Encircled characters indicate correspondence between scheme and subsequent figure panels. **(b)** Bath application of 0.5 μM TTX blocked both dendritic and axosomatic action potentials evoked by a 5-ms dendritic current pulse (950 pA). The recording site on the apical dendrite is 164 μm from the soma. **(c,d)** Local application of TTX to the dendrite near the recording pipette tip selectively blocked the dendritic spike, but left the axosomatic action potential unaffected. Responses to current pulses with three different intensities (400 pA, 700 pA and 1100 pA) are shown overlayed. Black traces represent somatic voltage and red traces represent dendritic voltage. Note that the dendritic action potential was blocked, whereas the axosomatic action potential was largely unaffected. This confirms the local nature of the application. The recording site on the apical dendrite is 209 μm from the soma. **(e,f)** Bath application of 200 μM Cd²⁺ did not affect dendritic spikes. Responses to current pulses with three different intensities (300 pA, 550 pA and 800 pA) are shown overlayed. Black traces represent somatic voltage and red traces represent dendritic voltage. The recording site on the apical dendrite is 263 μm from the soma. Note that Cd²⁺ only had negligible effects on dendritic spikes, suggesting that the contribution of Ca²⁺ channels is minimal. Scale bars also apply to **c** and **e**.

Figure 7.

Dendritic spikes increase the efficacy of axosomatic action potential initiation. **(a)** Schematic illustration of the recording configuration. Encircled characters indicate correspondence between scheme and subsequent figure panels. **(b)** Dendritic spikes evoked by EPSC-like current waveforms in CA3 pyramidal neurons. Top, EPSC-like currents were used as stimuli. The peak currents were 150, 600, 1,200, 1,800 and 2,400 pA. Center, corresponding EPSP-like waveforms. Bottom, expanded view of the somatic EPSP-like waveforms. Black traces represent somatic signals and red traces represent dendritic signals. The recording site on the apical dendrite is 142 μm from the soma. **(c)** Local application of 1 μM TTX to the dendrite prolonged the rising phase of the somatic EPSP-like voltage waveform and increased the initiation threshold of axosomatic spikes. EPSC-like current waveform; peak current was 150 pA and 300–2,100 pA in 300-pA increments. The recording site on the apical dendrite is 294 μm from the soma. Data in **b** and **c** were from different cells. Arrows indicate acceleration of rising phase produced by dendritic spikes. **(d)** Dendrosomatic propagation of dendritic spikes. Amplitude of dendritic spikes (DS) at the soma, normalized to that at the dendrite, was plotted against distance. The amplitude of the dendritic spike was measured after subtraction of scaled subthreshold responses. The attenuation of the backpropagated action potential (bAP) is replotted for comparison. **(e)** Summary graph of the average 20–80% rise time of EPSP-like voltage waveforms recorded at the soma without (–) or with dendritic spikes (+; $*P < 0.05$). **(f)** Summary graph of the average current threshold for the initiation of axosomatic spikes under control conditions and after local application of 1 μM TTX to the dendrite ($*P < 0.05$). In **e** and **f**, circles and lines represent data from individual experiments and bars indicate mean $\pm$ s.e.m.