ACTIVE PROPERTIES OF HIPPOCAMPAL CA3 PYRAMIDAL NEURON
DENDRITES

by

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Abstract

CA3 pyramidal neurons are important for memory formation and pattern completion in the hippocampal network. These neurons receive multiple excitatory inputs from numerous sources. Therefore, the rules of spatiotemporal integration of multiple synaptic inputs and propagation of action potentials are important to understand how CA3 neurons contribute to higher brain functions at cellular level. By using confocally targeted patch-clamp recording techniques, we investigated the biophysical properties of rat CA3 pyramidal neuron dendrites. We found two distinct dendritic domains critical for action potential initiation and propagation: 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 evoked by local dendritic depolarization or waveforms mimicking synaptic events. These findings can be explained by a high Na\(^+\)-to-K\(^+\) conductance density ratio of CA3 pyramidal neuron dendrites. The results challenge the prevailing view that proximal mossy fiber inputs activate CA3 pyramidal neurons more efficiently than distal perforant inputs by showing that the distal synapses trigger a different form of activity represented by dendritic spikes. The high probability of dendritic spike initiation in the distal area may enhance the computational power of CA3 pyramidal neurons in the hippocampal network.
Introduction

Figure 1 Dentate and CA hippocampal regions and their excitatory pathways. Adapted from de Almeida et al. (2007)

1.1 The hippocampal network
In 1971, O'Keefe and Dostrovsky (O'Keefe and Dostrovsky, 1971) reported that the rat hippocampus is involved in encoding spatial information and hippocampal neurons are candidates for the brain's spatial receptive field. Since then, their discovery has been supported by considerable experimental evidences and theoretical studies (Milner et al., 1998; Squire et al., 2004). The prevailing view is that synaptic transmission and plasticity in the hippocampus are responsible for learning and memory (Lisman and Otmakhova, 2001). The hippocampus is composed of three subregions, namely the CA3 region, the CA1 region, and the dentate gyrus (Lorente de Nó, 1934). Each subregion contains a large population of principal neurons and many type of interneurons. Those three subregions are connected in series via excitatory synapses,
forming the trisynaptic loop. The entorhinal cortex projects to dentate gyrus granule cells and the axons of these cells are targeted to CA3 pyramidal neurons. The axons of the CA3 pyramidal cells are terminated in CA1 pyramidal neurons, which provide the output of the hippocampal network back to the cortex. Therefore, understanding the cellular mechanisms of spatial information processing in the hippocampal network requires detailed biophysical knowledge of each type of hippocampal principal neurons.

1.2 CA3 pyramidal neurons

CA3 network is critical for pattern completion and error correction (McNaughton and Morris, 1987; Lisman, 1999; Nakazawa et al., 2002, 2004; Marr, 1971). To understand the cellular mechanisms underlying CA3 network functions, knowledge about anatomical connectivity and intrinsic properties of CA3 pyramidal neurons is essential. These neurons receive three different glutamatergic inputs (Fig. 1, dashed box), whose targeting synapses are well segregated on their apical dendrites. In the proximal dendrites, mossy fibers from dentate gyrus granule cells connect to large thorny excrescences of dendrites and efficiently discharge the CA3 pyramidal cells (Pelkey and McBain, 2005; Henze et al., 2002). In the distal dendrites, perforant path synapses arising from the entorhinal cortex may relay contextual information (Lisman, 1999) or the spatiotemporal information generated by grid cells in the entorhinal cortex (Hafting et al., 2005). However, how distal synaptic signals are conducted to the soma via the long dendritic cable has not been resolved. In the intermediate dendrites, commissural / associational (C/A) synapses between CA3 cells are thought to store memories by spike timing-dependent plasticity, but whether backpropagated action potentials efficiently invade the postsynaptic dendrites at CA3 pyramidal neurons is unclear (Bi and Poo, 1998; Magee and Johnston, 1997; Debanne et al., 1998). To unravel how these three complex pathways can interact, it is essential to study the biophysical properties of CA3 pyramidal neuron dendrites.

1.3 The passive and active properties of dendrites

Dendrites are extremely thin cellular processes to receive synaptic inputs and exhibit a wide range of morphological properties (Fig. 2). In the early era of dendritic research,
dendrites were regarded as merely passive cables. In 1950s, Wilfrid Rall assumed that dendrites are only electrical compartments consisting of resistive and capacitive properties. He studied how synaptic conductances at different dendritic locations influences action potential generation (Rall, 1959). One important finding by his early work was that synaptic efficacy depends on the electrotonic distance of the synapse from the soma due to dendritic filtering. Following the first direct intracellular recording from a dendrite (Llinas and Nicholson, 1971), more and more evidence has been accumulated suggesting that synaptic signals are reshaped by the dendritic active conductances in addition to dendritic passive filtering. However, direct dendritic patch-clamp recording is limited to a few types of neurons with thick dendrites (Stuart and Sakmann, 1994; Spruston et al., 1995b). These measurements showed that the intrinsic biophysical properties of the dendrites are highly diverse. Thus, to understand how
information is processed by each type of neurons, detailed knowledge about both passive and active integrative properties of dendrites is needed.

1.3.1 Passive properties
The passive properties of the dendrite include its morphological characteristics and three additional cable parameters describing the membrane properties of the dendrites: the specific membrane resistivity \( R_m \), the specific membrane capacitance \( C_m \), and the intracellular resistivity \( R_i \). \( C_m \) is dependent on membrane thickness and composition. \( R_m \) is more widely used in its inverse form, which is known as the leak conductance \( G_m = R_m^{-1} \). \( R_i \) is influenced by the presence of intracellular organelles which restrict charge redistribution within neurons (Koch, 1999). Although the values of \( C_m \) are similar among different types of neuron, the values of \( R_m \) and \( R_i \) are highly diverse (Stuart and Spruston, 1998; Golding et al., 2005; Major et al., 1994; Nörenberg et al., 2010). The basic concept of electrical signal propagation within thin neuronal processes like dendrites is based on the cable theory, which was first developed by Lord Kelvin to describe signal decay in underwater telegraph cables (Jack et al., 1983). The attenuation of a steady-state voltage signal along an infinite passive cable can be described as:

\[
\lambda = \sqrt{\frac{R_m}{r_i}} = \sqrt{\frac{R_m d}{R_i / 4}}
\]

The space constant \( \lambda \) indicates the spatial distance, where the steady state voltage decreases to \( e^{-1} \) (37% of its original value). Variables with capital letters are values independent of the of cable diameter \( d \) (Koch, 1999). The attenuation of electrical signals along a passive cable is also frequency dependent. The space constant of a frequency dependent electrical signal can be defined as:

\[
\lambda(f) = \frac{\lambda(0)}{\text{Re}\{\sqrt{1 + i2\pi f \tau_m}\}}
\]

The membrane time constant \( \tau_m \) is defined as the time it takes for the membrane potential to increase or to decrease to \( 1 - e^{-1} \) (63% of its final value), and calculated by the membrane resistance \( (r_m) \) and the membrane capacitance \( (c_m) \), \( \tau_m = r_m \cdot c_m \). \( \lambda(0) \) is the space constant in response to a steady current (Eisenberg and Johnson, 1970).
\( \lambda(f) \) decays with increasing frequency \( f \). Thus, passive cables behave like low-pass filters, with fast electrical events, i.e. action potentials, being attenuated more than slow events (Koch, 1999). Although the above equation provides an accurate description of the spread of electrical signals within a simple cable-like structure, the propagation of electrical signals in real neurons are far more complicated because dendrites and axons differ from a simple infinite cable by having complex branch patterns. For example, when an action potential arrives at the branch points, propagation failure can occur due to the mismatch of the electrical load between parent and daughter branches. The theoretical study by Goldstein and Rall (1974) indicates that the propagation of action potential past the bifurcation depends on the geometric ratio (GR):

\[
GR = \frac{d_{\text{daughter},1}^{3/2} + d_{\text{daughter},2}^{3/2}}{d_{\text{parent}}^{3/2}}
\]

For \( GR \leq 1 \), reliable propagation into the daughter dendrite is assured. Whereas if \( GR > 10 \), the action potential fails to propagate into both branches. By using the morphology from realistic neurons, Vetter et al. (2001) theoretically studied the impact of dendritic geometry on action potential backpropagation along the dendrite. They found that the efficacy of action potential propagation is largely affected by the number of branch points in the dendritic tree. Action potentials are severely attenuated during propagation in those neurons with extensive dendritic branches, like cerebellar Purkinje neurons. Interestingly, the structure of the dendritic tree can not only affect the propagation of action potentials, can but also have a strong impact on the intrinsic firing patterns independent with differential channel expression and distribution or other physiological mechanisms like intracellular Ca\(^{2+}\) dynamics (Mainen and Sejnowski, 1996).

### 1.3.2 Active properties

The active properties of dendrites have been the focus of dendritic research for the last fifteen years. It is now clear that the dendrites of many neurons differ from passive cables by having active conductances, which allow these dendrites to perform complex nonlinear computations. For instance, dendritic voltage-gated Na\(^+\) and Ca\(^{2+}\) channels promote the backpropagation of action potentials along the dendrite and can also trigger local dendritic action potentials within the dendrite (Stuart and Sakmann, 1994;
Spruston et al., 1995b; Martina et al., 2000; Bischofberger and Jonas, 1997; Spruston, 2008). By contrast, dendritic voltage-gated K\(^+\) channels suppress action potential backpropagations and the initiation of dendritic action potentials (Hoffman et al., 1997). The distribution of dendritic active conductance is highly diverse among different types of neurons (Hoffman et al., 1997; Korngreen and Sakmann, 2000; Stuart et al., 2007). Furthermore, any active conductances along the dendrite are often nonuniformly distributed even within the same neuron (Hoffman et al., 1997; Magee, 1999). For instance, the densities of A-type K\(^+\) channels and H-channels are several folds higher in the distal dendrites of CA1 pyramidal neurons than in the soma (Hoffman et al., 1997; Magee, 1999). The high densities of those channels in distal dendrites suppress the synaptic efficacy and narrow the integration time window of the synaptic inputs to distal dendrites of CA1 pyramidal neurons (Hoffman et al., 1997; Magee, 2000). Furthermore, the gating properties of dendritic active channels are often nonuniform along the proximodistal axis. For instance, voltage-gated Na\(^+\) channels on the distal dendrite of CA1 pyramidal neurons have a slower recovery rate from inactivation than the somatic voltage-gated channels, which regulate action potential backpropagation in a frequency-dependent manner (Colbert et al., 1997; Jung et al., 1997). Such a frequency-dependent backpropagation is known to be accompanied by a reduced Ca\(^{2+}\) influx (Spruston et al., 1995b) and might have important functional implications during spike timing-dependent plasticity (STDP; Letzkus et al., 2006). Thus, the nonuniform distribution of active conductances and their gating properties along the dendrite greatly enhance the computational power of neurons.

1.4 Backpropagating action potentials (bAPs)
When synaptic inputs are integrated and finally exceed the threshold for axonal spike initiation, AP output is evoked in the axosomatic region. Then APs travel through the axon fiber and relay information to the next target neurons. Simultaneously, APs propagate back into the dendrites, providing a feedback signal to the dendrites (Stuart et al., 1997). Those feedback signals, termed bAPs, play a variety of roles during neuron network activity. First, bAPs are important for STDP (Bi and Poo, 1998; Magee and Johnston, 1997; Markram et al., 1997). When backpropagating action potentials in
the postsynaptic neuron are paired with the synaptic input from the presynaptic neuron, the dendritic depolarization induced by bAPs will relieve the blockade of NMDA receptors by Mg²⁺ and thus modify the strength of the synapse. STDP has been extensively studied both in vivo and in vitro (Zhang et al., 1998; Kobayashi and Poo, 2004). Interestingly, blocking bAPs in vivo by downregulating the voltage-gated Na⁺ channels in the somatodendritic domain of layer 2 and 3 neurons interferes with experience-dependent synaptic development in barrel cortex (Komai et al., 2006). Second, bAPs regulate the output pattern of the neuron through its interaction with local excitatory postsynaptic potential (EPSP). On the apical dendrite of neocortical layer 5 pyramidal neurons, pairing bAP and synaptic input amplifies the local dendritic depolarization by 3 to 4 fold. Bursting firing occurs when the local dendritic depolarization propagates to the soma and subsequently promotes the afterdepolarization following the action potentials (Larkum et al., 1999; Stuart and Häusser, 2001). Bursting firing is known to be more reliably transmitted to the downstream neurons at the output synapse than a single action potential (Lisman, 1997). Thus, bAP might increase efficacy of the network by promoting bursting firing through its interaction with excitatory synaptic inputs on the dendrite. Third, bAP is associated with the release of neurotransmitters from dendrodendritic synapses (Xiong and Chen, 2002; Margrie et al., 2001; Casale and McCormick, 2011). The extent of backpropagation is highly diverse among CNS neurons due to the heterogeneities in their passive and active dendritic properties (Stuart et al., 2007; Vetter et al., 2001; Bischofberger and Jonas, 1997; Martina et al., 2000; Stuart et al., 1997; Stuart and Sakmann, 1994). For example, in dopamine neurons of the substantia nigra, somatically evoked APs show nondecremental attenuation with a distance from the soma, whereas markedly attenuated dendritic APs with increasing distance were shown in cerebellar Purkinje cells (Häusser et al., 1995; Stuart and Häusser, 1994). Most of the dendrites display the attenuation level between these extremes, supporting their different rules of decremental propagation. These findings again demonstrate the diversity of the intrinsic excitability of neurons, and emphasize the importance of careful measurements from each type of neurons in order to understand the crucial factors governing the information processing in neurons.
1.5 Dendritic spikes
The presence of dendritic active conductances implies that neurons have multiple action potential initiation zones, which might have a significant impact on the computational capacity of the neuron network. Indeed, locally initiated dendritic action potentials have been observed in the apical and basal dendrite of many neuronal types with direct dendritic patch-clamp recordings (Stuart et al., 2007). Glutamate-uncaging and Ca\textsuperscript{2+}-imaging experiments also suggested that oblique or basal dendrites are capable of supporting active action potential backpropagation and initiation of dendritic action potentials, although direct patch-clamp recordings have not been obtained from these small caliber dendrites yet (Losonczy and Magee, 2006; Schiller et al., 2000). There are several different types of active conductances mediating dendritic spikes; fast spikes mediated by Na\textsuperscript{+} channels, slow spikes mediated by Ca\textsuperscript{2+} channels, and N-methyl-D-aspartate (NMDA) receptor-mediated spikes (Llinas and Sugimori, 1980; Golding and Spruston, 1998; Nevian et al., 2007; Larkum et al., 1999; Schiller et al., 2000). Dendritic spikes have different impacts on neuronal processes. First, dendritic spikes in distal synapses are able to compensate the strong attenuation of the distal synaptic inputs (Larkum and Zhu, 2002). Second, local dendritic spikes can boost the efficacy and speed of generation of axosomatic action potentials (Ariav et al., 2003). Those spikes are coupled with all-or-none accelerated somatic responses, which enable neurons to have a short-latency action potential output to any following input from perisomatic synapses (Gasparini et al., 2004; Jarsky et al., 2005). Third, dendritic spikes are also known to implement a mechanism for local submillisecond coincidence detection and multiplicative computations in dendrites (Softky, 1994; Poirazi and Mel, 2001). Traditionally, plasticity and associativity have long been known to occur in synapses between coactive neurons. However, recent work revealed that active dendrites can modify local excitability, and in collaboration with somatic spikes function as input feature detectors (Losonczy et al., 2008; Larkum and Nevian, 2008). This may increase the memory capacity of single pyramidal neurons (Poirazi and Mel, 2001). Fourth, dendritic spikes may induce non-Hebbian plasticity (Golding et al., 2002). These heterosynaptic forms of plasticity can be especially effective in small-diameter branches at the distal dendrites or the basal dendrites (Lisman and Spruston, 2005), because
bAP often fail to invade the distal dendritic tuft in several types of neurons (Golding et al., 2002). In conclusion, dendrites of a variety of different neuronal types are equipped with different machinery for the initiation of local spikes, which is also different in a distance dependent manner, suggesting that individual neuron types have different computational strategies for information processing.

1.6 The purpose of this study
The hippocampal CA3 region is essential for a wide range of memory-related mechanisms such as spatial memory formation and pattern completion. However, the active properties of CA3 pyramidal neurons are still poorly characterized, I performed confocally targeted patch clamp recordings from dendrites and axons with somatic whole-cell recordings to address the following question:

1) Do CA3 pyramidal neuron dendrites support active bAP?
2) Where is the site of action potential initiation?
3) Do CA3 pyramidal neurons have a single integration zone or multiple integration zones?
4) What is the biophysical mechanism underlying these electrical properties?
Methods

2.1 Brain dissection and cutting of slices

Transverse hippocampal slices (thickness, 350 µm) were prepared from the brains of 24- to 29-day-old Wistar rats of either sex. Animals 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). The skin was removed rapidly and the skull was opened with a single sagittal cut from caudal to frontal. Immediately after opening the skull, the head was gently dipped into ice-cold cutting solution and the brain was always kept in cold saline during dissection. The hemispheres were separated by a sagittal cut through the corpus callosum and only one hemisphere was isolated from the base of the skull with a spatulum and transferred into fresh cutting solution. The total duration of procedures until this stage was taken within 1 minute. The hemisphere was put on a Sylgard-coated Petri dish, with the sagittal plane down and the temporal surface up. The dorsal part of the brain was cut away with the different angles (Bischofberger et al., 2006). The cutting angles with slicing direction will roughly determine the target area (CA3 a,b,c). To mount the hemisphere, the plate of the buffer tray of the slicer was wiped carefully and coated with a thin layer of cyanoacrylate glue. All solutions were equilibrated with a carbogen gas mixture (95% O₂ and 5% CO₂) using microfilter candles.

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 room temperature (Bischofberger et al., 2006; Davie et al., 2006; Hu et al., 2010). Slices were then individually transferred into a recording chamber perfused with standard physiological saline. Recordings were performed at room temperature (~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.
2.2 Subcellular patch-clamp recording

To obtain recordings from dendrites and axons of CA3 pyramidal neurons, the following experimental strategy was adopted (Nevian et al., 2007; Larkum et al., 2009; Hu et al., 2010). First, a somatic recording configuration was obtained, using an internal solution containing the fluorescent dye, 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, Perkin Elmer, equipped with an Orca camera, Hamamatsu, and an argon / krypton laser; excitation wavelength 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-DIC (Hu et al., 2010). Axonal recordings were obtained at axon blebs, artificial enlargements formed by the slicing procedure near the surface of the slice (Shu et al., 2006; Kole et al., 2007; Schmidt-Hieber et al., 2008).

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 cell-attached patch recording, typically pipettes with resistance of 9–23 MΩ were used. For comparison of channel density between soma and dendrites (Fig. 8), pipettes had similar size and geometry. For cell-attached recordings, pipettes were coated with dental wax to reduce capacitance (Pluradent, Offenbach, Germany). 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 used for the Figures of the present thesis).

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, Freiburg) running under Igor Pro 6.21 or 6.22 (WaveMetrics). To assess the active components of action potential backpropagation, an action potential was recorded in the current-clamp mode, digitized, and subsequently applied as voltage-clamp command at the soma in the same cell, using FPulse. In this set of experiments, correction and prediction circuits of the amplifier 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 (Spruston et al., 1995a; Jonas et al., 1993), was injected into the dendrite. For dynamic clamp experiments, we used a system that was either based on a digital signal processor card (Lien and Jonas, 2003) or an analog circuit 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 R_s 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 typically the series resistance was the lowest.

2.3 Outside-out patch-clamp recording
To obtain excised outside-out patch-clamp recording from soma and dendrites, the pipette is carefully pulled away from the cell after establishing the whole-cell configuration. Broken membrane attached around the pipette will reseal to form a new patch. After obtaining outside-out patches, the resistance of the feedback resistor was increased to reduce the noise level of the recording. To record voltage-gated Na⁺ current in outside-out patches, a pulse sequence comprised of a 100-ms prepulse to
–120 mV and a 30-ms test pulse to 0 mV was applied. To measure voltage-gated K+ currents, 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 was applied. 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.

2.4 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 shock-frozen 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 lucidum (Chicurel and Harris, 1992). Conversely, CA3 pyramidal neuron axons were unequivocally identified by the lack of spines.

2.5 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. Tetrodotoxin (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 sec and amplitudes of ~10 psi. Cd\(^{2+}\) was applied in the bath at a concentration of 200 \(\mu\)M (this concentration was saturating for Ca\(^{2+}\) channel block, but below that severely affecting A-type channels (Song et al., 1998)). TTX was purchased from Biotrend, tetraethylammonium (TEA) and 4-aminopyridine (4-AP) were from Sigma-Aldrich.

For whole-cell recording and K\(^+\) current recording in outside-out patches, a K\(^+\)-rich internal solution was used, which contained 135 mM K-gluconate and 0.1 mM EGTA, or 120 mM K-gluconate and 10 mM EGTA, 20 mM KCl, 2 mM MgCl\(_2\), 2 mM Na\(_2\)ATP, 0.2\% biocytin, and 10 mM HEPES, pH adjusted to 7.3 with KOH. 100 \(\mu\)M Alexa Fluor 488 was added 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 Cs-gluconate, 20 mM CsCl, 0.1 or 10 mM EGTA, 2 mM MgCl\(_2\), 2 mM Na\(_2\)ATP, 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 CaCl\(_2\), 1 mM MgCl\(_2\), 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 CaCl\(_2\), 1 mM MgCl\(_2\), 2.5 KCl, 1 \(\mu\)M TTX, 25 mM D-glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH.

2.6 Data analysis
Analysis was performed using Igor Pro 6.21 or 6.22 (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 measured using the time points at half-maximal amplitude in the action potential rising phase. To determine the action potential initiation
site, action potential latencies were fit with a 3\textsuperscript{rd} 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 
\[ f(V) = A \left[ 1 + \text{Exp}\left(\frac{V - V_{0.5}}{k}\right) \right]^{-1} + B, \]
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 relation (Sakmann and Neher, 1995). Distances were measured linearly from the center of the soma to the tip of the dendritic or axonal recording pipette resulting in underestimation of the actual distance measured trajectorially. Also, it should be noted that distance measurements were made from the center of the soma, not from its origin on the soma. 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.
Results

To assess the electrical properties of CA3 pyramidal neurons, improved subcellular patch-clamp recording techniques were used (Bischofberger et al., 2006; Nevian et al., 2007; Larkum et al., 2009; Hu et al., 2010) (Fig. 3). Dendritic recordings were made at a distance of up to 403 µm from the soma (Nevian et al., 2007; Larkum et al., 2009; Hu et al., 2010) (Fig. 3a–c; 23 recordings in stratum oriens, 37 in stratum lucidum, 134 in stratum radiatum, and 4 in stratum lacunosum-moleculare; Table 1). Axonal measurements were obtained up to 151 µm from the soma (Shu et al., 2006) (Fig. 3d–f).

3.1 Action potential initiation and backpropagation

We first used subcellular recording to determine the site of action potential initiation and the rules of propagation (Fig. 4). To achieve this, simultaneous somatodendritic and axosomatic recordings were performed. Rheobase current pulses (that is long current pulses near threshold) applied at the soma initiated low-frequency trains of action potential propagation.

Table 1 Width of morphologically defined layers in the hippocampal CA3 region.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum lucidum</td>
<td>93 ± 3 µm</td>
</tr>
<tr>
<td>Associational zone (~inner 4/5) of stratum radiatum</td>
<td>176 ± 4 µm</td>
</tr>
<tr>
<td>Stratum radiatum (estimated)</td>
<td>220 ± 5 µm</td>
</tr>
<tr>
<td>Stratum lacunosum-moleculare</td>
<td>231 ± 12 µm</td>
</tr>
</tbody>
</table>

The width of the different layers was measured in 10 – 12 biocytin-labeled CA3 pyramidal neurons. Stratum lucidum was recognized as the region densely populated with thorny excrescences. The associational zone (~inner 4/5) of stratum radiatum was identified as the region with multiple oblique dendritic side branches (Ishizuka et al., 1995). The width of stratum lacunosum-moleculare was measured from the boundary of stratum radiatum to the tip of the dendrites of CA3 pyramidal cells. Values indicate mean ± s.e.m.
Figure 3  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 µM Alexa Fluor 488 via the somatic recording pipette during the experiment is shown in a. The dendritic recording site is 218 µm 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 µM Alexa Fluor 488, showing an axon bleb at the surface of the slice, is presented in d. The axonal recording site is 90 µm 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 4 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 \((dV/dt)_{\text{max}}\) (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.
potentials in CA3 pyramidal neurons (Fig. 4a); 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 typical for CA3 pyramidal cells (Spruston and Johnston, 1992). Simultaneous somatodendritic recording revealed that the action potential evoked by somatic current pulses was first detected at the somatic and later at the dendritic recording sites (Fig. 4a, bottom left). In contrast, simultaneous axosomatic recording showed that the action potential in the proximal axon preceded the somatic waveform (Fig. 4a, bottom right). This temporal sequence was highly consistent from trial to trial and among cells, suggesting robust axonal action potential initiation followed by dendritic backpropagation (Stuart et al., 2007).

Next, we examined the distance dependence of peak amplitude and duration of the dendritic action potential in CA3 pyramidal neurons (Fig. 4b,c). Intriguingly, the peak action potential amplitude declined sigmoidally as a function of distance from the soma, with little change within the first 100 µm (Fig. 4b; n = 43 somatodendritic recordings; Table 1). On average, the amplitude of the action potential at the dendrite between 50 and 100 µm was 91 ± 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 in the first 100 µm (Fig. 4c). On average, the half-duration of the action potential at the dendrite between 50 and 100 µm was 106 ± 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, action potential latency and maximal rate of rise \( (\text{dV/dt})_{\text{max}} \) were plotted against distance (Fig. 4d,e). Analysis of the data set 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 (Colbert and Johnston, 1996; Meeks and Mennerick, 2007). 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 \( \text{Na}^+ \) conductance in this region. In conclusion, these results suggest that during application
Figure 5 Action potential initiation and propagation at near-physiological temperature.

(a) Train of action potentials evoked in a CA3 pyramidal cell by somatic current injection. Current intensity 275 pA. (b) Left, first action potential evoked by a 1-s current pulse applied to the soma as shown in a. Right, dendritic spike evoked by a 5-ms current pulse applied to the dendrite in a different cell (1.3 nA). Recording site on apical dendrite 141 µm and 116 µm from the soma, respectively. Black traces, somatic voltage and corresponding current; red traces, dendritic voltage and corresponding current. Recording temperature in a, b ~33°C. (c–e) Summary plot of action potential peak amplitude measured from threshold (c), maximal slope of rise (d), and duration at half-maximal amplitude (e) plotted against distance (positive distance, apical dendrite; negative distance, basal dendrite; both measured from the center of the soma). Data at near-physiological temperature (~33°C, blue symbols, 6 simultaneous somatodendritic recordings) are overlayed with the data obtained at ~22°C (43 simultaneous somatodendritic recordings; data replotted from Fig. 4; symbol code identical to that in Fig. 4b,c).

Of somatic rheobase stimuli the action potential was initiated in the proximal axon and subsequently propagated back into the dendrites. Similar results were obtained at ~22°C and at near-physiological temperature (~33°C; Fig. 5).
Figure 6 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.

CA3 pyramidal neurons fire bursts of action potentials (typically 3–5) under a variety of conditions both in vitro and in vivo (Traub and Miles, 1991). To examine whether action potential backpropagation was activity dependent, CA3 pyramidal neurons were stimulated repetitively with trains of brief somatic current pulses (Fig. 6). Trains of pulses were applied at frequencies of 20, 50, and 100 Hz (Fig. 6a–c). After the fifth action potential of trains at 100 Hz, brief somatic current pulses occasionally failed to elicit action potentials. Therefore, the properties of the fifth action potential were
compared with those of the first action potential for both somatic and dendritic recording sites. Action potential 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. 6d). Likewise, 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. 6e). 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 was caused by cable properties of the neurons (Jaffe and Carnevale, 1999) or by active conductances, I 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 µM tetrodotoxin (TTX) were compared with naturally backpropagating action potentials (Fig. 7). Dendritic peak amplitudes of passively propagated action potentials were significantly smaller than those of naturally propagated action potentials in the same cells, showing that voltage-gated Na⁺ channels provided a substantial contribution to active dendritic action potential propagation (Fig. 7a,b,d; 7 somatodendritic recordings; P < 0.05). In contrast, control experiments with two pipettes both located at the soma gave voltage signals with similar amplitude (5 dual somatic recordings; P > 0.2), confirming the validity of the experimental approach (Fig. 7c).

3.2 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. 8). 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. 8a). These currents were blocked by 0.5 µM extracellular TTX, indicating that they were mediated by voltage-gated Na⁺ channels. Under conditions in which voltage-gated K⁺ currents were
Figure 7 Dendritic Na\(^+\) channels mediate active action potential backpropagation in CA3 pyramidal neurons.

(a) Natural dendritic propagation of action potentials. Simultaneous recording from soma (black) and dendrite (red) of a CA3 pyramidal neuron, both in the current-clamp configuration (CC). Recording site on apical dendrite 92 µm from the soma. Note that the peak amplitude of the dendritic action potential (red) was similar to that of the somatic action potential (black). (b) Passive dendritic propagation of action potential-like voltage signals. The somatic action potential recorded previously in the same cell (black) was used as a command signal in the voltage-clamp configuration (VC) and applied to the soma in the presence of 0.5 µM TTX, while the resulting voltage change was measured at the dendrite. Note that the dendritic action potential (red) was substantially attenuated. Data in (a, b) were taken from the same cell. (c) Control experiment using a double-somatic recording. The action potential wave was applied as voltage-clamp command with one pipette, and the somatic voltage was recorded under current-clamp conditions with the second pipette in the presence of 0.5 µM TTX. (d) Plot of action potential amplitude against distance. Black circles, somatic action potentials recorded under CC conditions; red circles, dendritic action potentials under CC conditions (both in the absence of TTX); open circles, dendritic action potentials in waveclamp experiments in the presence of 0.5 µM TTX. Note that the naturally propagated dendritic action potential had a markedly larger amplitude than the passively propagated action potential under waveclamp conditions in the presence of TTX, showing the active nature of action potential backpropagation (positive distance, apical dendrite; negative distance, basal dendrite; zero distance, soma).
Figure 8 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. A residual outward current presumably represents a Cs\(^+\) current through Kv3-type K\(^+\) channels. (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).
Table 2 Current and conductance densities of Na\(^+\) and K\(^+\) channels in somata and dendrites of CA3 pyramidal neurons.

<table>
<thead>
<tr>
<th></th>
<th>Na(^+) channels</th>
<th>A-type K(^+) channels</th>
<th>Delayed rectifier K(^+) channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current density (pA µm(^{-2})), soma</td>
<td>26.3 ± 3.3 (n = 12)</td>
<td>16.5 ± 1.9 (n = 12)</td>
<td>34.1 ± 3.8 (n = 12)</td>
</tr>
<tr>
<td>Current density (pA µm(^{-2})), dendrite</td>
<td>16.2 ± 1.1 (n = 41)</td>
<td>28.6 ± 2.9 (n = 38)</td>
<td>20.8 ± 2.5 (n = 38)</td>
</tr>
<tr>
<td>Conductance density (mS cm(^{-2})), soma</td>
<td>35.0</td>
<td>10.0</td>
<td>20.6</td>
</tr>
<tr>
<td>Conductance density (mS cm(^{-2})), dendrite</td>
<td>21.6</td>
<td>17.3</td>
<td>12.6</td>
</tr>
<tr>
<td>Spearman rank correlation coefficient</td>
<td>0.40</td>
<td>0.45</td>
<td>0.16</td>
</tr>
<tr>
<td>Significance P</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

For calculation of dendritic current and conductance density, data from apical and basal dendrites were pooled.
For calculation of conductance, reversal potentials were assumed as 75 mV for Na\(^+\) currents (Martina and Jonas, 1997) and −95 mV for K\(^+\) currents (Martina et al., 1998). Values indicate mean ± s.e.m.

voltage-gated Na\(^+\) channels. Under conditions in which voltage-gated K\(^+\) currents isolated (that is with a K\(^+\)-rich intracellular solution and test pulses to +70 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. 8b,c). To separate the components, we applied a pulse protocol with two different prepulses (−120 mV and −40 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 mM extracellular 4-AP (Fig. 8b), but not by 20 mM TEA (not illustrated). In contrast, a delayed rectifier current component, which was
isolated using depolarizing prepulses, was largely suppressed by 20 mM extracellular TEA (Fig. 8c).

To analyze the spatial distribution of Na\(^+\), A-type K\(^+\), and delayed rectifier K\(^+\) current components, current density was plotted against distance from the soma (Fig. 8d–f). Intriguingly, 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 (Fig. 8d; Spearman rank correlation analysis; n = 41 dendritic outside-out patches; P < 0.01). In contrast, the dendritic A-type K\(^+\) current density increased monotonically from the soma to the distal dendritic region (Fig. 8e; n = 38 dendritic outside-out patches; P < 0.01). Finally, the delayed rectifier K\(^+\) current density was not significantly dependent on distance (Fig. 8f; P > 0.1). Conversion of dendritic current density into conductance density revealed that the average ratio of Na\(^+\) to total K\(^+\) conductance density was 0.72 (Table 2). Thus, CA3 pyramidal neuron dendrites show a high Na\(^+\) to K\(^+\) current ratio in comparison to other types of neurons (Stuart and Sakmann, 1994; Hu et al., 2010). To confirm the results of the outside-out patch recordings, cell-attached recordings were performed and comparable results were obtained (Fig. 9). 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 (Fig. 10). 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; Table 3).

3.3 Dendritic spikes in CA3 pyramidal neurons

The high dendritic Na\(^+\) channel density together with the more negative activation threshold of dendritic Na\(^+\) channels raises the possibility that CA3 pyramidal neurons may generate dendritic spikes (Golding and Spruston, 1998; Kamondi et al., 1998; Golding et al., 2002; Gasparini et al., 2004; Losonczy et al., 2008). Indeed, short current pulses applied to the dendrite evoked dendritic spikes highly efficiently (Fig. 11). Dendritic spikes occurred in complete isolation with stimuli slightly above threshold, but
Figure 9 High Na\(^+\) to K\(^+\) conductance ratio and distinct conductance gradients in dendritic cell-attached patches.

(a) Na\(^+\) current (average from 116 – 124 single traces) evoked in cell-attached patches from soma and apical dendrite of CA3 pyramidal cells (163 \(\mu\)m). In addition to the cell-attached electrode, a somatic whole-cell configuration was established for dye loading and control of membrane potential. Test pulse potential 0 mV. Na\(^+\) current was recorded with 20 mM TEA and 3 mM 4-AP in the recording electrode. Left, soma; right, dendrite. Leak and capacitive currents were subtracted by a ‘P over –8’ or ‘P over –4’ correction procedure. (b) A-type K\(^+\) current (average from 82–94 single traces) evoked in cell-attached patches from soma and apical dendrite (176 \(\mu\)m). A-current was isolated by subtraction of traces with a –40-mV prepulse from those with a –120-mV prepulse. Left, soma; right, dendrite. Pulse protocols in b and c: Prepulse to –120 mV or –40 mV, test pulse to 70 mV. Data in b and c were taken from the same patch. Current traces recorded in the cell-attached configuration were inverted to facilitate comparison with outside-out patch data. (d–f) Plot of Na\(^+\) current density (d), A-type K\(^+\) current density (e), and delayed rectifier K\(^+\) current density (f) against distance (positive distance, apical dendrite; negative distance, axon). Data from 7, 2, and 2 somatic (black circles), 15, 8, and 8 dendritic (red circles), and 2 axonal cell-attached patches (blue circles).
Figure 10 Gating properties of dendritic and somatic Na\(^+\) and K\(^+\) channels in CA3 pyramidal neurons.

(a) Na\(^+\) current (average from 10 single traces each) evoked in an outside-out patch from apical dendrite of CA3 pyramidal cells (133 µm). Activation protocol: Prepulses to −120 mV, test pulse potentials from −70 to +20 mV in 10 mV steps. Inactivation protocol: Prepulses from −120 mV to −30 mV in 10 mV steps, test pulse 0 mV. Na\(^+\) current was recorded with Cs\(^+\)-internal solution. Upper panel, activation; lower panel, inactivation. Leak and capacitive currents were subtracted by a ‘P over –8’ correction procedure. (b) A-type K\(^+\) current (average from 3–5 single traces each) evoked in outside-out patch from apical dendrite (148 µm). Activation protocol: Prepulses to −40 mV or −120 mV, test pulse potentials from −60 to +50 mV in 10 mV steps. Inactivation protocol: Prepulses from −120 mV to −10 mV in 10 mV steps, test pulse to +70 mV. 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. (c) Delayed rectifier K\(^+\) current (average from 3 single traces each) evoked in outside-out patch from apical dendrite (120 µm). Activation protocol: Prepulse to −40 mV, test pulse potentials from −60 to +50 mV in 10 mV steps. Delayed rectifier K\(^+\) current was isolated by a −40-mV prepulse. (d–f) Corresponding activation and inactivation curves for Na\(^+\) current (d), A-type K\(^+\) current (e), and delayed rectifier K\(^+\) current (f). Circles, activation curves; squares, inactivation curves. Black symbols and lines, somatic patches; red symbols and lines, dendritic patches. Conductance values were normalized to the maximal value. Data points indicate mean ± s.e.m. Data were fitted with a Boltzmann function, with midpoint potentials and steepness factors given in Supplementary Table 3. Results from 6 (activation), 6 (inactivation), 6 (activation), 6 (inactivation), and 6 (activation) somatic outside-out patches and 6, 7, 5, 5, and 6 dendritic outside-out patches. Recordings in b, c, e, and f were obtained in the presence of 0.5 µM TTX to block Na\(^+\) channels. Midpoint potentials of activation curves were significantly different between dendrite and soma for Na\(^+\) and A-type K\(^+\) current, but not delayed rectifier K\(^+\) current (P < 0.01; P < 0.05; P > 0.5).
Table 3 Gating properties of Na\(^+\) and K\(^+\) channels in somata and dendrites of CA3 pyramidal neurons

<table>
<thead>
<tr>
<th>Location</th>
<th>Parameter</th>
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<th>A-type K(^+) channels</th>
<th>Delayed rectifier K(^+) channels</th>
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<tbody>
<tr>
<td>Soma</td>
<td>Activation midpoint potential</td>
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<td>–5.1 mV</td>
<td>–5.1 mV</td>
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<td></td>
<td>Activation slope factor</td>
<td>6.1 mV</td>
<td>14.4 mV</td>
<td>12.1 mV</td>
</tr>
<tr>
<td></td>
<td>Inactivation midpoint potential</td>
<td>–73.5 mV</td>
<td>–63.4 mV</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Inactivation slope factor</td>
<td>7.9 mV</td>
<td>5.8 mV</td>
<td>N/A</td>
</tr>
<tr>
<td>Dendrite</td>
<td>Activation midpoint potential</td>
<td>–37.6 mV</td>
<td>–9.6 mV</td>
<td>–3.8 mV</td>
</tr>
<tr>
<td></td>
<td>Activation slope factor</td>
<td>7.9 mV</td>
<td>16.0 mV</td>
<td>10.2 mV</td>
</tr>
<tr>
<td></td>
<td>Inactivation midpoint potential</td>
<td>–72.9 mV</td>
<td>–65.6 mV</td>
<td>N/A</td>
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<tr>
<td></td>
<td>Inactivation slope factor</td>
<td>8.0 mV</td>
<td>7.1 mV</td>
<td>N/A</td>
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</table>

Gating parameters were determined by fitting data points in Fig. 9 with Boltzmann functions.

were coupled to subsequent axosomatic spikes with larger stimuli (Fig. 11a). 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. 11b; Fig. 12). Analysis of voltage-current relations revealed that dendritic spikes were all-or-none events and often occurred in isolation from axosomatic spikes (Fig. 11c). The probability of
dendritic spike initiation in CA3 pyramidal cells was surprisingly high, reaching a value close to 1 for distances > 100 µm (Fig. 11d; P < 0.001).

It is generally thought that distal synapses are much less efficient in activating CA3 pyramidal neurons than proximal synapses, due to filtering of synaptic potentials along the dendritic cable (Rall, 1977; Spruston, 2008). I therefore analyzed the distance dependence of axosomatic and dendritic spike threshold (Fig. 10e,f). For axosomatic spikes, the current threshold increased significantly as a function of distance, as expected from cable theory (Fig. 11e; P < 0.05). For dendritic spikes, however, the threshold decreased substantially with distance (Fig. 11f; P < 0.001), suggesting that a small number of unitary inputs may be sufficient for initiation. Similar results were obtained with multiexponential conductance waveforms applied in the dynamic clamp configuration (Fig. 12). The two threshold curves crossed at a distance of ~125 µm (Fig. 11e,f). Thus, CA3 pyramidal neurons show two distinct functional dendritic domains. In the proximal domain, action potentials backpropagate with nearly constant amplitude and time course and EPSCs trigger somatic spikes. In contrast, in the distal domain EPSCs initiate dendritic spikes with a low threshold. Thus, activity of a small number of converging inputs may be sufficient to trigger dendritic spikes in CA3 pyramidal neurons.

3.4 Ionic mechanisms and functional impact

To identify the ion channels underlying dendritic spikes, I tested the effects of blockers of voltage-gated Na\(^+\) and Ca\(^{2+}\) channels (Fig. 13). Global application of 0.5 µM TTX in the bath blocked both axosomatic and dendritic spikes (3 out of 3 cells; Fig. 13a,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 out of 4 cells; Fig. 13c,d). This suggests that the Na\(^+\) channels 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 out of 6 cells; Fig. 13e,f). Taken together, these results indicate that dendritic spikes are mediated by voltage-gated Na\(^+\) channels, rather than Ca\(^{2+}\) channels.
Figure 11 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 and 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 12** Dendritic spikes initiated by artificial synaptic conductances generated by a dynamic clamp.

(a) Schematic illustration of the dynamic clamp system. An exponentially rising and decaying conductance waveform \( G(t) \) was generated by the computer. The membrane potential \( V(t) \) was measured by the dendritic electrode. The corresponding current \( I(t) \) was calculated by a digital signal processor (DSP) or an analog dynamic clamp system \( I(t) = G(t) \times (V(t) - V_{\text{rev}}) \), where \( V_{\text{rev}} \) is reversal potential) and injected into the dendrite. (b) Dendritic spikes evoked by synaptic event-like conductance \( G(t) \) waveforms. Recording site on apical dendrite 146 µm from the soma. Top, multiexponential conductance waveforms used as stimuli. The peak conductance was increased from 1.2 nS to 12 – 60 nS in 12 nS steps. Bottom, corresponding EPSP-like voltage waveforms. Black traces, somatic signals; red traces, dendritic signals. (c) Plot of initiation conductance threshold for dendritic spikes plotted against distance (positive distance, apical dendrite; negative distance, basal dendrite). Note that the conductance threshold of dendritic spike initiation decreases as function of distance, similar to the current threshold (**Fig. 11f**).
Figure 13 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 in d, f also apply to c, e, respectively.
Figure 14 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 was 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 was 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 ± s.e.m.
Figure 15 Less efficient action potential backpropagation and stronger activity dependence in CA1 as compared to CA3 cell dendrites.

(a) CA1 pyramidal neuron visualized by biocytin labeling and post-hoc DAB staining (stack projection). (b, c) Action potential backpropagation in CA1 pyramidal neurons during somatic injection of long current pulses or a 100-Hz train of brief current pulses. Dendritic recording site 430 µm and 275 µm from the soma. (d–f) Summary bar graph of proximal dendritic action potential peak amplitude normalized to that at the soma (d), proximal dendritic action potential half-duration normalized to that at the soma (e), and amplitude of the 5th dendritic action potential in a 100-Hz train, normalized to that of the first dendritic action potential (f) in CA3 (gray bars) and CA1 pyramidal neurons (open bars). Circles represent data from individual experiments and bars indicate mean ± s.e.m. (**P < 0.01, *P < 0.05, and *P < 0.05, respectively). (g–i) Plot of action potential peak amplitude (g), action potential half-duration (h), and amplitude of the 5th action potential, normalized to that of the first action potential (i) in CA1 pyramidal neurons against distance (zero distance, soma). In g, h, somatic rheobase current stimuli were used. In i, trains of brief pulses at different frequencies were applied (red circles, 20 Hz; black triangles, 50 Hz; blue squares, 100 Hz). The ratio of action potential amplitude of the fifth action potential over that of the first action potential was in the range of 0.8–0.1, markedly lower than in CA3 pyramidal cells. Dashed curves represent a fitted exponential function (g) or the results of linear regression (h, i).
To determine the impact of dendritic spikes on axosomatic output of CA3 pyramidal neurons, I examined the effect of dendritic spikes evoked by synaptic event-like currents and conductances on the somatic voltage (Fig. 14a–c). Dendritic spikes attenuated substantially during propagation from the dendrites to the soma (Fig. 14d). 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 EPSP (Fig. 14b), 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. 14c,f).

Comparison of the present results in CA3 pyramidal neurons with published data on CA1 pyramidal neurons (Spruston et al., 1995b; Hoffman et al., 1997; Golding and Spruston, 1998; Gasparini et al., 2004) suggests that dendritic properties differ between the two types of neurons. To demonstrate these differences directly under identical conditions, we performed similar experiments in CA1 pyramidal neuron dendrites (Figs. 15 and 16). Our results demonstrate that in CA1 pyramidal neurons (1) action potential backpropagation in the proximal dendrite shows significantly larger attenuation, broadening, and activity dependence (Fig. 15), (2) dendritic Na\(^+\) channel density is lower and more uniform (Fig. 16b,c), and (3) dendritic spikes are evoked with lower probability (Fig. 16d,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.
Figure 16 Smaller dendritic \( \text{Na}^+ \) current density and markedly lower probability of dendritic spike initiation in CA1 as compared to CA3 cell dendrites.

(a) CA1 pyramidal neuron visualized by biocytin labeling and post-hoc DAB staining (stack projection; same cell as in Fig. 15). (b) \( \text{Na}^+ \) current (average from 40 single traces) evoked in an outside-out patch from apical dendrite of a CA1 pyramidal neuron (181 µm). Test pulse potential 0 mV. \( \text{Na}^+ \) current was recorded with Cs\(^+\)-internal solution. Leak and capacitive currents were subtracted by a ‘P over –8’ correction procedure. (c) Plot of \( \text{Na}^+ \) current density against distance from the soma in CA1 pyramidal neuron dendrites. Data from 7 somatic and 9 dendritic patches (blue circles). Data from CA3 pyramidal neurons are replotted for comparison (black and red circles). (d) Examples of dendritic spikes in CA1 pyramidal neurons. Top trace, 5-ms current pulses (intensity: 960 pA and 1240 pA). Middle trace, subthreshold response. Bottom trace, dendritic spike, followed by axosomatic spike. Black traces, somatic signals; red traces, dendritic signals. Dendritic recording site 274 µm from the soma. (e) Histogram of number of recordings with dendritic spikes (gray bars) and without dendritic spikes (open bars) in CA1 pyramidal neurons at different distances from the soma. Blue continuous curve shows the corresponding probability of dendritic spike initiation (right axis), as obtained by fitting with a Boltzmann function. Data for CA3 pyramidal neurons are replotted for comparison (red dashed line).
Discussion

This thesis presents the first analysis of the electrical properties of dendrites in CA3 pyramidal neurons. The analysis demonstrates that there are functional differences between proximal dendrites and distal dendrites of the CA3 pyramidal neurons, which receive differential inputs from the mossy fibers of granule cells and the perforant pathways from the entorhinal cortex, respectively. Together with previous results (Spruston et al., 1995b; Krueppel et al., 2011), these data suggest that different types of principal cells within the trisynaptic hippocampal circuit differ substantially in their dendritic properties (Table 4).

4.1 Comparison with dendrites in other neuron types
The present results reveal several similarities, but also differences in dendrites of other types of pyramidal neurons, especially CA1 pyramidal neurons (Spruston et al., 1995b). 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) where action potential backpropagation is only moderately decremental and associated with minimal changes in time course. In contrast, in CA1 pyramidal neurons action potential amplitude declines exponentially and action potential duration increases as a function of distance (Spruston et al., 1995b) (Fig. 15). 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 neurons (Jaffe et al., 1992; Spruston et al., 1995b). Thus, in CA3 neurons bAPs 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 patterns (Bi and Poo, 1998; Magee and Johnston, 1997; Debanne et al., 1998).

4.2 Dendritic spikes in CA1 and CA3 pyramidal neurons
The specific dendritic properties also lead to unique characteristics of local electrogensis. In CA3 neurons, dendritic spikes were observed with a probability of
Table 4 Multiple differences between dendrites of CA3 pyramidal neurons and those of the two other hippocampal principal neurons.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CA3 pyramidal neurons</th>
<th>CA1 pyramidal neurons</th>
<th>References</th>
<th>DG granule cells *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+) conductance density versus distance</td>
<td>Increasing</td>
<td>Constant (this paper)</td>
<td>Magee &amp; Johnston, 1997</td>
<td>Low</td>
</tr>
<tr>
<td>I(_A) conductance density versus distance</td>
<td>Moderately increasing</td>
<td>Markedly increasing</td>
<td>Hoffman et al., 1997</td>
<td>Low</td>
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<tr>
<td>Delayed rectifier conductance density versus distance</td>
<td>Decreasing</td>
<td>Constant</td>
<td>Hoffman et al., 1997</td>
<td>Unknown</td>
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<td>Action potential backpropagation, dendritic action potential amplitude</td>
<td>Only moderately decremal in the first 100 µm</td>
<td>Exponentially decreasing</td>
<td>Spruston et al., 1995b</td>
<td>Markedly attenuating</td>
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<td>Action potential backpropagation, dendritic action potential time course</td>
<td>Nearly constant time course in the first 100 µm</td>
<td>Broadening</td>
<td>Spruston et al., 1995b</td>
<td>Nearly constant time course</td>
</tr>
<tr>
<td>Action potential backpropagation, activity dependence</td>
<td>Minimal</td>
<td>Yes</td>
<td>Spruston et al., 1995b; Jaffe et al., 1992</td>
<td>Minimal</td>
</tr>
<tr>
<td>Dendritic spike initiation</td>
<td>96% of neurons for dendritic recordings &gt; 100 µm</td>
<td>~30% of neurons</td>
<td>Golding &amp; Spruston, 1998</td>
<td>Absent</td>
</tr>
<tr>
<td>Dendritic spike initiation, current threshold</td>
<td>~500 pA (&gt; 300 µm)</td>
<td>3.9 nA</td>
<td>Gasparini et al., 2004</td>
<td>N/A</td>
</tr>
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* All data in this column from Krueppel et al., 2011.
near 1 at a distance of > 100 µm 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 (Golding and Spruston, 1998; Gasparini et al., 2004) (Fig. 16). 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 (Holmes, 1989; Jaffe and Carnevale, 1999), 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 dendritic Na\(^+\) channel activation curve. In contrast, CA1 pyramidal cells have less branch points, the increase of A-type K\(^+\) channel density, and the uniform distribution of Na\(^+\) conductance along the dendrites. 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 (Fig. 17).

4.3 Functional implications for synaptic input and AP output
The functional properties of dendrites of CA3 pyramidal neurons have major implications for both the input and the output of CA3 pyramidal neurons. At the level of input, their morphological characteristics in proximal dendrites of CA3 pyramidal neurons may assist the conditional detonator function of mossy fiber synapses (Henze et al., 2002). It is well known that presynaptic factors such as a large number of release sites and a large size of the releasable pool of synaptic vesicles contribute to the proposed detonator function of mossy fiber synapses (Chicurel and Harris, 1992; Hallermann et al., 2003; Rollenhagen et al., 2007). Postsynaptically, CA3 proximal dendrites have a thick diameter trunk with thorny excrescences, which may increase the probability of mossy fiber EPSPs to contribute to reach the threshold for axonal spike initiation without filtering loss. At the level of output, the enrichment of dendrites of CA3 cells with Na\(^+\) channels play a role in the afterdepolarization (ADP) following spikes. In
Figure 17 An active cable model of a CA3 pyramidal neuron reproduces the spatial profile of AP backpropagation and the distal initiation of dendritic spikes.

(a) Top, dendrogram; center, somatic AP (black) and backpropagated action potential (red) at distal dendritic segment (#135, 622 µm from the soma) evoked by 5-ms current pulses. Bottom, plot of action potential latency against distance from the soma. Note that minimal latency is found in the axon 81 µm from the soma, representing the AP initiation site. (b) Top, plot of action potential peak amplitude; center, half-duration; bottom, maximal rate of rise against distance. (c) Initiation of dendritic spikes by synaptic conductances. Top, original traces (soma, black; dendrite, red); middle, plot of peak dendritic response against peak synaptic conductance. The positive nonlinear deflection was used to determine the threshold of dendritic spiking. Bottom, plot of threshold of dendritic spike initiation against distance. Note that the threshold declines as a function of distance. Simulations were performed on a Neuron 7.1 platform. Detailed cable model of a CA3 pyramidal neuron, taken from Jonas et al., 1993 and Major et al., 1994. Cell CA3_15. Passive parameters: $R_i = 190 \ \Omega \text{cm}$, $R_m = 100000 \ \Omega \ \text{cm}^2$, $C_m = 0.8 \ \mu \text{F cm}^2$. Active parameters: Hodgkin-Huxley $\text{Na}^+$ and $\text{K}^+$ channels, $g_{\text{Na}}$ (soma) = 15 mS cm$^{-2}$, $g_{\text{Na}}$ (dendrite) = 15 mS cm$^{-2}$, $g_{\text{Na}}$ (axon) 30 – 60 mS cm$^{-2}$. $g_K = 10$ mS cm$^{-2}$. 


CA3 pyramidal neurons, several factors in channel distribution and kinetics contribute to the ADP, which may be the mechanism inducing burst firing in these cells. First, our data show high Na\(^+\)-to-K\(^+\) conductance ratio, the dendrosomatic axial current flow is enhanced by Na\(^+\) inflow, leading to a substantial and long-lasting depolarization of the neuron following a spike (Wong and Prince, 1981). Second, the Na\(^+\) channel activation curve shows a hyperpolarized shift in the dendrite, while inactivation range is not significantly different. It also supports the idea that dendrites can employ more inward currents, resulting in the increase of probability of the ADP. Third, delayed rectifier K\(^+\) current shows the decreasing density with distance from the soma, contributing to the ADP over afterhyperpolarization (AHP). A very different situation is found in fast-spiking GABAergic interneurons (Hu et al., 2010). In dentate gyrus basket cells, in which the dendrites contain a high density of Kv3-type potassium channels, the dendrosomatic axial current flow after an action potential is reduced by K\(^+\) efflux, or even converted into an AHP (Hu et al., 2010). The difference in membrane time constant (50 – 100 ms in CA3 pyramidal neurons versus ~10 ms in fast-spiking GABAergic interneurons; Spruston and Johnston, 1992; Major et al., 1994; Nörenberg et al., 2010) between the two cells will further contribute to the functional consequences, generating a slow decay of the afterpotential in CA3 pyramidal cells, but a faster decay in interneurons.

4.4 Function of the dendritic spikes in CA3 pyramidal neurons

On the basis of cable theory, it is generally assumed that proximal mossy fiber synapses activate CA3 pyramidal cells more powerfully than distal synapses (Rall, 1977; Major et al., 1994; Pelkey and McBain, 2005). 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, fast dendritic spikes may accelerate the efficacy and enhance the temporal precision of axosomatic action potentials (Ariav et al., 2003). Second, dendritic spikes that can be evoked by temporally synchronous and spatially clustered inputs in a branch-specific manner can enlarge the input
sensitivity of a neuron, thereby increasing the memory capacity of single CA3 pyramidal neurons (Softky, 1994; Poirazi and Mel, 2001). Third, dendritic spikes will shape synaptic plasticity rules, relieving the Mg$^{2+}$ block of NMDA receptors (Golding et al., 2002; Kampa et al., 2004). 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 (McMahon and Barrionuevo, 2002; Kobayashi and Poo, 2004). Finally, dendritic spikes will help to process spatially and temporally precise grid cell input from the entorhinal cortex via the perforant path (Urban et al., 1998; Urban and Barrionuevo, 1998; Hafting et al., 2005). Lisman (1999) suggested that subthreshold contextual information from the entorhinal cortex via distal synapses promotes dendrites more depolarized, enabling a single mossy fiber finally detonating the CA3 cell. However, the present data indicate that small number of synaptic inputs can produce local dendritic spikes in the distal dendrites. These dendritic spikes may implement a form of heterosynaptic plasticity 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.

4.5 Functional implications for synaptic plasticity and memory storage

Previous models have assumed that mossy fiber synapses induce postsynaptic spiking activity (Henze et al., 2002), which can trigger STDP in commissural/associational CA3–CA3 cell synapses (Kobayashi and Poo, 2004). However, one problem with this assumption is timing. If mossy fiber activation drives a postsynaptic spiking, and a postsynaptic spiking in turn activates CA3-CA3 cell synapses, then activity sequence at these synapses will be a post-pre sequence. This sequence is likely to lead to the induction of LTD, rather than LTP (Bi and Poo, 1998). It is difficult to see how storage of patterns would take place under these conditions. However, our results suggest a different model of memory storage. According to our results, activation of perisomatic mossy fiber synapses will facilitate the bidirectional transfer of charge between dendrites and soma. Mossy fiber activation and the associated dendritic depolarization
will enhance the amplitude of local EPSPs generated at distal excitatory synapses by promoting the recruitment of active conductances. With this facilitated transfer, incomplete input patterns will be sufficient to generate suprathreshold EPSPs. Finally, dendritic spikes will trigger the induction of LTP directly, independently of the initiation of somatic APs. All these factors will converge towards the facilitation of the induction of LTP, leading to a conversion of labile memories corresponding to incomplete active ensembles into stable memories corresponding to robust active ensembles.

4.6 Impact of inhibition in CA3 pyramidal neurons

GABAergic interneurons innervate CA3 pyramidal neurons in a subcellular domain-specific manner (Freund and Buzsáki, 1996). Diverse classes of interneurons can selectively provide feedback or feedforward inhibition to the principal cells to coordinate their activity patterns in both space and time. Perisomatic interneurons by basket or axoaxonic cells may control timing of spike generation of CA3 pyramidal neurons (Pouille and Scanziani, 2001; Cobb et al., 1995). In the distal area of CA3 neurons, dendrite-targeting interneurons may increase dendritic spike thresholds or suppress the forward propagation of dendritic spike, which promote burst spiking (Lovett-Barron et al., 2012). Alternatively, it is possible that inhibition can regulate local membrane excitability by increasing the availability of Na\(^+\) conductance. Additional Na\(^+\) channels could become available during hyperpolarizing inhibition, resulting in a transient increase in local excitability.

4.7 Comparison of action potential initiation site in other neuron types

Our latency analysis reveals that the primary site of action potential initiation in CA3 pyramidal neuron is the proximal part of the axon, approximately 75 \(\mu\)m from the center of the soma. Consistent with this idea, the \(dV/dt_{\text{max}}\) data also suggest that maximal Na\(^+\) conductance is located near this region. These results show that the AP initiation zone in CA3 pyramidal neurons is located more distally from the soma than that of other cortical neurons. It is interesting to note that CA3 pyramidal neurons have the longest axon initial segment throughout the nerve cells. However, it may be difficult to compare our distance measurements with those of other pyramidal neurons. Our distance
measurement is made from the tip of the axonal recording to the center of the soma whereas in other reports, the reference point for measurement and the exact way of distance measurement (linear vs trajectorial) is often not specified. Alternatively, the proximal axon has to charge a large membrane capacitance of the soma and the dendrite therefore the distal region may be favored for AP initiation (Mainen et al., 1995). In addition, it should be noted that axonal recordings were obtained from axon blebs where the cytoskeleton is presumably disrupted (Kole et al., 2008). Therefore, we cannot exclude that AP initiation site could be shifted.
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tr>
<td>ADP</td>
<td>afterdepolarization</td>
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<tr>
<td>AHP</td>
<td>afterhyperpolarization</td>
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<tr>
<td>AMPAR</td>
<td>$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor</td>
</tr>
<tr>
<td>bAP</td>
<td>backpropagating action potential</td>
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<tr>
<td>BC</td>
<td>basket cell</td>
</tr>
<tr>
<td>C/A</td>
<td>commissural / associational</td>
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<td>cornu ammonis region 1</td>
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<tr>
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2003–2006    M.A. in Cognitive and Neural Systems
              Boston University, Boston, MA, USA
1995–2002    B.S. in Biochemistry
              Yonsei University, Seoul, Korea
              (This period included 26 months of mandatory military service)
**RESEARCH EXPERIENCE**

2008-Present  
Laboratory of Dr. Peter Jonas, Institute of Science and Technology (IST) Austria, Klosterneuburg, Austria (2011-Present)  
University of Freiburg, Freiburg, Germany (2008-2010)

2005-2008  
Laboratory of Dr. Jen-Wei Lin, Department of Biology, Boston University, Boston, MA, USA

**TEACHING EXPERIENCE**

2006-2008  
Teaching Assistant, Department of Biology, Boston University, Boston, MA, USA  
Principle of Neuroscience, Spring & Summer 2008  
Techniques in Cellular and Molecular Neuroscience, Fall 2006/2007  
Systems Physiology, Spring 2007  
(taught three weekly discussion sections; wrote and graded weekly quizzes; graded exams, laboratory reports and papers; held weekly office hours; proctored exams)

**CONFERENCE PRESENTATION**


**Kim S** and Jonas P (2010) Action potential initiation and dendritic propagation in hippocampal CA3 pyramidal neurons. Oral presentation at the SFB/TR3 Junior Scientists' 2nd annual meeting, Bonn, Germany

**Kim S** and Lin JW (2006) Two inhibitory synapses colocalized within the same terminal: A comparison on their calcium dependence of transmitter release and short term plasticity. Poster presentation at the Boston Univ. Science and Engineering Research Symposium, Boston, MA, USA
Kim S and Lin JW (2005) Serotonergic modulation of high and low output inhibitory synapses at the crayfish neuromuscular junction. Poster presentation at the Boston Univ. annual meeting of Biology dept., Boston, MA, USA

**PUBLICATION**

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Finally, I would like to dedicate this work to my family and friends in my home country for always supporting and encouraging me with their best wish.
Appendix

Video:

“Confocally targeted subcellular patch-clamp recordings from dendrites of CA3 pyramidal neuron”

Publication: