Dendritic Spikes Are Enhanced by Cooperative Network Activity in the Intact Hippocampus

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In vitro experiments suggest that dendritic fast action potentials may influence the efficacy of concurrently active synapses by enhancing Ca$^{2+}$ influx into the dendrites. However, the exact circumstances leading to these effects in the intact brain are not known. We have addressed these issues by performing intracellular sharp electrode recordings from morphologically identified sites in the apical dendrites of CA1 pyramidal neurons in vivo while simultaneously monitoring extracellular population activity. The amplitude of spontaneous fast action potentials in dendrites decreased as a function of distance from the soma, suggesting that dendritic propagation of fast action potentials is strongly attenuated in vivo. Whereas the amplitude variability of somatic action potentials was very small, the amplitude of fast spikes varied substantially in distal dendrites. Large-amplitude fast spikes in dendrites occurred during population discharges of CA3–CA1 neurons concurrent with field sharp waves. The large-amplitude fast spikes were associated with bursts of smaller-amplitude action potentials and putative Ca$^{2+}$ spikes. Both current pulse-evoked and spontaneously occurring Ca$^{2+}$ spikes were always preceded by large-amplitude fast spikes. More spikes were observed in the dendrites during sharp waves than in the soma, suggesting that local dendritic spikes may be generated during this behaviorally relevant population pattern. Because not all dendritic spikes produce somatic action potentials, they may be functionally distinct from action potentials that signal via the axon.

Key words: plasticity; calcium spikes; long-term potentiation; spike propagation; signaling; action potentials

Experiments using extracellular techniques, intracellular recordings with sharp electrodes, and patch-clamp pipettes have led to competing but not necessarily mutually exclusive scenarios regarding the initiation and active propagation of action potentials (Johnston et al., 1996; Yuste and Tank, 1996; Stuart et al., 1997). According to one view, fast (Na$^+$) action potentials are initiated in the axon, and active dendritic Na$^+$ conductances facilitate their back-propagation into the dendritic tree (Turner et al., 1991; Jaffe et al., 1992; Stuart and Sakmann, 1994; Magee and Johnston, 1995; Spruston et al., 1995). Actively back-propagating fast action potentials may affect the efficacy of concurrently active synapses and may influence synaptic plasticity by enhancing Ca$^{2+}$ influx into the cell (Yuste and Denk, 1995; Christie et al., 1996; Jester et al., 1996; Magee and Johnston 1997; Markram et al., 1997). An alternative view is that strong excitatory synaptic activation may lead to action potentials generated within the dendrites independent of the soma or axon initial segment (Spencer and Kandel, 1961; Llinás and Nicholson, 1971; Wong et al., 1979; Herreras 1990; Turner et al., 1991; Wong and Stuart, 1992; Regehr et al., 1993). Because most experiments in this area of research have been done in the slice preparation, the argument can be made that the amount of depolarization produced by electrical stimulation of presynaptic fibers for the demonstration of dendritic spike initiation is artificial, and such synchrony never exists under physiological conditions (Mainen et al., 1995; Buzsáki et al., 1996; Miles et al., 1996; Tsubokawa and Ross, 1996). It is therefore important to investigate the rules of dendritic electrogenesis and its modification by physiologically relevant network events in the intact brain (Swoboda et al., 1997).

In the behaving animal, two physiologically antagonistic population patterns are known in the hippocampus: theta waves and sharp waves (Buzsáki et al., 1983). During theta activity, somata of pyramidal cells, in general, are hyperpolarized and rarely fire (Leung and Yim, 1986; Fox, 1989; Soltesz and Dechénes, 1993; Ylinen et al., 1995b). In contrast, pyramidal cells are depolarized during sharp waves (SPW) (Ylinen et al., 1995a) and often fire “complex-spike” bursts (Spencer and Kandel, 1961; Ranck, 1973). SPWs are large-amplitude (1–3 mV) aperiodic field potentials (40–100 msec) observed in stratum radiatum of the CA1 region (Buzsáki, 1986, 1989; Suzuki and Smith, 1987) present during awake immobility, consummatory behaviors, and slow-wave sleep. Field SPWs result from the excitation of the dendritic fields of CA1 pyramidal cells and interneurons by their CA3 Schaffer collateral input. The ramp-like depolarization of CA1 neurons induces a dynamic interaction between interneurons and pyramidal cells, the result of which is a short-lived oscillatory field potential (ripple) within stratum pyramidale and a phase-related discharge of the CA1 network at 200 Hz (Buzsáki et al., 1992;
Ylinen et al., 1995a). In association with field SPWs, ~40,000–
60,000 cells discharge in concert in the CA3–CA1–subiculum–
presubiculum–layer V entorhinal cortex axis (Chrobak and
Buzsáki, 1996). In summary, the SPW burst is the most powerful
depolarizing population pattern in the hippocampal formation.
We reasoned therefore that if active properties of dendrites are
critically influenced by synaptic inputs, then their physiological
relevance in the intact brain could be investigated during the
occurrence of SPW-associated population bursts.

MATERIALS AND METHODS

Surgery and recording. Ninety-three rats of the Sprague Dawley strain
(250–350 gm) were anesthetized with urethane (1.3–1.5 gm/kg) and
placed in a stereotaxic apparatus. The body temperature of the rat
was kept constant by a small-animal thermoregulation device. The scalp
was removed, and a small (1.2 × 0.8 mm) bone window was drilled above the
hippocampus (anteroposterior at ~3.3 mm from anteromedial edge and
lateral at 2.2 mm from bregma) for extracellular and intracellular record-
ings. The cisterna magna was opened, and the CSF was drained to
prevent drying of the brain and to decrease pulsation.

In vivo pH 7.2, containing 2% biocytin for intracellular labeling. In vivo elec-
trode impedances varied from 60 to 100 MΩ. Once stable, intracellular
recordings were obtained and evoked, and passive physiological proper-
ties of the cell were determined. Only neurons with a resting potential
more negative than ~55 mV were included in this study. Because the
resting membrane potential fluctuates in vivo, we used the voltmeter
readings of the amplifier (Axoclamp-2B; Axon Instruments, Foster City,
CA) to obtain an average value integrated over time. Input resistance
of the neurons varied between 17 and 67 MΩ. Field activity recorded
through the extracellular electrode was filtered between 1 Hz and 5 kHz.
The extracellular activity and the extracellular field potential or the
extracellular field potential were digitized at 10 kHz with 12 bit precision (ISC-16 board; RC Electronics,
Santa Barbara, CA). The electrophysiological data were stored on optical
disks. In three rats, bicuculline meth-CI (5 mg/kg) was injected intra-
peritoneally after baseline recordings (~15 min), and recordings contin-
ued for 30–140 min.

Data analysis. The data were analyzed off-line. The extracellular trace
was digitally filtered at 120 dB/octave to separate the fast-ripple waves
(50–200 Hz) and unit activity (500 Hz–5 kHz). Cross-correlograms
between intracellular spikes and extracellular unit activity were per-
formed using a selected amplitude or pattern of intracellular spikes as
reference signals. Extracellular units were detected by amplitude dis-
riminator software. For the amplitude measurements of intracellular
fast spikes, the extracellular trace was filtered (50 Hz–5 kHz) to eliminate
synaptic potentials and slow spikes. These discriminated spikes were used
to construct amplitude histograms, interspike interval histograms, and
waveform averages using the original unfiltered traces. Spike amplitude,
rate of rise, rate of decay, amplitude of spike afterpotential, and half-
width were determined for each recorded neuron.

Histological analysis. After the completion of the physiological data
collection, biocytin was injected through a bridge circuit using 500 msec
depolarizing pulses at 0.6–2 nA at 1 Hz for 10–60 min. Neuronal activity
was followed throughout the procedure, and the current was reduced if
the electrode was blocked and/or the condition of the neuron deterior-
rated. Two to 12 hr after the injection, the animals were given a urethane
overdose and then perfused intracardially with 100 ml of physiological
saline followed by 400 ml of 4% paraformaldehyde and 0.2% glutaral-
dehyde dissolved in PBS, pH 7.2. The brains were then removed and
stored in the fixative solution overnight. Sixty- or 100-µm-thick coronal
sections were cut and processed for biocytin labeling (Sik et al., 1995).
The labeled neurons were reconstructed with the aid of a drawing tube.
Double- and multiple-labeled neurons were discarded from the analysis.
The histological sections were also used to verify the position of the
extracellular recording electrodes and the track made by the recording
pipette. Both physiological and histological methods were used to local-
ize the tip of the recording electrode. During the experiment the micro-
meter readings of the microstepper (Inchworm; Burleigh Inc., Fishers,
NY) indicated the depth from the pyramidal layer. The pyramidal layer
was recognized by the high density of impaled somata. After the with-
drawal of the pipette from the dendrite, extracellular averaged evoked
responses to commissural stimulation were obtained at 50 µm steps from
the recording site to the pyramidal layer. The polarity reversal of the
extracellular field response at the border of strata radiatum and pyrami-
dale provided an additional landmark for the pyramidal layer. The
location of dendritic penetration was determined from the distance
between the pyramidal layer and the recording site (measured in a
straight line) during the experiment and from the anatomical reconstruc-
tion of the electrode track and the dendritic tree of the filled neuron.

RESULTS

Properties of dendritic action potentials vary with distance from soma

All recordings in this study were made from biocytin-injected and
morphologically identified CA1 pyramidal cells. Double- and
multiple-labeled neurons were discarded from the analysis. Stable
measurements (30 min–4 hr; resting membrane potential less
than ~55 mV) were made from 56 dendrites at distances of up to
400 µm from the pyramidal layer. Somatic recordings were avail-
able from 28 additional neurons. Somatic recordings were char-
acterized by short (0.98 ± 0.09 msec half-amplitude width) on-
shooting action potentials with little amplitude variance,
undershooting spike afterhyperpolarization, frequency of spon-
taneous firing <2 Hz, and the lack of slow spikes (see below) in response
to depolarizing pulses.

During the experiment, the location of the recorded dendrite was
estimated from the travel distance of the recording pipette from the
pyramidal layer. In addition, after the withdrawal of the
pipette from the dendrite the extracellular averaged evoked re-
sponses to commissural stimulation were obtained at 50 µm steps from
the site of dendritic impalement to the pyramidal layer. The
polarity reversal of the extracellular field response at the border of
strata radiatum and pyramidaile provided a clearcut landmark
for the pyramidal layer (Andersen et al., 1971). Anatomical
verification of the recording track was available in 26 cases. In
several instances, the exact location of the impalement could also
be verified from the recording track and the reconstructed den-
dritic tree (Fig. 1A). Occasionally, bulging of the dendrite or the presence of biocytin-filled astrocytic processes further assisted
the determination of the location of dendritic impalement.

Recordings from apical dendrites of CA1 pyramidal cells rep-
licated several features of dendritic action potentials described in
vitro (Wong et al., 1979; Wong and Stuart, 1992; Spruston et al.,
1995; Tsubokawa and Ross, 1996). The amplitude of fast action
potentials was always smaller than that recorded from the soma
(Fig. 2A). Depolarizing pulses in dendrites evoked a train of
action potentials with progressively decrementing amplitude. The
recordings shown in Figure 1 were obtained from the distal part
of a primary dendritic shaft in the outer third of stratum radia-
Low-intensity current steps (0.4 nA) induced a moderate spike frequency adaptation and amplitude decrement of the action potentials. Larger current steps induced a more complex pattern associated with three additional changes. First, a large-amplitude slow spike, likely reflecting high-threshold Ca\(^{2+}\) currents (Wong et al., 1979; Wong and Stuart, 1992; Magee and Johnston, 1995), was evoked. Second, the slow spikes were associated with a burst of fast spikes, one of which was often much larger in amplitude (Fig. 1B, arrow) than those evoked by smaller currents. Third, large-amplitude slow spikes were followed by...
suppression of fast spikes, likely attributable to activation of 
$\text{Ca}^{2+}$-mediated increase of $K^+$ conductance (Fig. 1) (Hotson and Prince, 1980; Schwarzkroin and Stafstrom, 1980). Synaptically 
evoked postsynaptic potentials in dendrites were of larger ampli-
tude, and the latency of evoked action potential varied more than 
in the soma. Action potentials could occur before or after the 
peak of the evoked EPSP (Fig. 1C).

In addition to the above differences, dendritic and somatic fast 
action potentials were different in several more respects. The amplitude of spontaneously occurring single action potentials 
decreased as a function of the distance from the soma (Fig. 2). Several other parameters of spontaneously occurring spikes, in-
cluding the rate of rise and decay, the width at half-amplitude, 
and the spike afterpotential, also correlated with the location of 
the dendritic recording (Fig. 2B). The undershooting spike after-
hyperpolarization observed in somatic recordings was absent or, 
in fact, was depolarizing in the dendrite. The resting membrane 
potential was slightly but significantly more hyperpolarized in the 
dendrites ($-65.2 \pm 2.2 \text{ mV}$) than in the soma ($-61.2 \pm 1.9 \text{ mV}$; 
$t = 3.26; p < 0.002$). However, the resting membrane potential did 
not change with distance from the soma.

The amplitude of spontaneous spikes in dendrites was rather 
constant from the soma up to $\sim 250 \text{ m\mu}$ from the somatic layer 
(Fig. 2A). This was followed by a step-wise amplitude decrement 
in the distal third of stratum radiatum. Regression lines fitted to 
the proximal-middle group ($< 280 \text{ m\mu}$) and distal group inter-
cepted the $y$-axis at 55 and 48 mV, respectively. However, the 
slopes of the linear regression lines were significantly different 
($F(6,30); p < 0.015$). In only one case was the spike amplitude $<15$ 
$\text{ mV}$ in the proximal two-thirds of stratum radiatum. In this single 
case (Fig. 2, triangle at 220 m\mu), the recordings were made from 
an anatomically verified second-order branch.

Network influence on dendritic spikes

A striking difference between somatic–proximal dendritic and 
distal dendritic recordings was the large range of spike amplitudes 
in distal dendrites. Although recordings at distal locations typically 
revealed small action ($<15 \text{ mV}$) potentials, large-amplitude

Figure 3. Sharp wave burst-induced amplitude enhancement of fast spikes. A, Reconstructed dendritic tree. The micropipette points to the anatomically verified penetrated dendrite. B, Responses to hyperpolarizing ($-0.5 \text{ nA}$) and depolarizing (left, 0.5 nA; right, 0.6 nA) current steps. Arrow, Large-amplitude fast spike (LAS); asterisk, putative calcium spike. C, Responses to commissural (c) stimulation. Note large-amplitude-evoked fast spike (arrow) and absence of spike (bottom trace) with and without concurrent direct depolarization of the dendrite, respectively. The same stimulus intensity was used in both cases. D, E, Relationship between extracellularly recorded multiple unit activity (MUA) and field ripples from CA1 pyramidal layer and intradendritic activity (dendrite). There is a 45 sec gap between traces in D and E. Cross-correlogram (burst vs MUA) between intradendritic bursts as defined by repeating spikes at $<10 \text{ msec}$, interspike intervals, and extracellular MUA activity illustrates that the incidence of intradendritic bursts was highest during ripple-related MUA. E, left inset, Large-amplitude fast spikes were present exclusively during MUA bursts. Note that the small potential associated with the large fast spike is larger than the stimulation-evoked EPSP shown in C. Right inset, Cross-correlogram between large-amplitude ($>30$ 
$mV$) spikes and MUA activity (LAS vs MUA). Ordinate, Number of units per bin.
(20–50 mV) spontaneous spikes were also observed as part of a burst (Fig. 3). When action potentials were evoked by intradendritic current pulses, the amplitude of the first few evoked spikes was often larger than that of the spontaneously occurring action potentials. Spikes evoked by suprathreshold afferent stimulation were also larger in amplitude than the spontaneously occurring spikes. This was in sharp contrast to somatic recordings, because the amplitude of evoked spikes in the soma was always 10–30% smaller than that of the spontaneously occurring action potentials, likely attributable to a shunting effect of IPSPs mediated by the feed-forward activation of basket cells (Sik et al., 1995; Miles et al., 1996). Subthreshold afferent stimulation combined with intradendritic current-induced depolarization often evoked large-amplitude spikes (Fig. 3). These findings indicated that local regeneration of fast spikes can be enhanced by depolarization of the dendrite.

After studying dendritic events in response to current injections and electrically evoked synaptic responses, we questioned whether the magnitude of dendritic depolarization required for these events is ever achieved under physiological conditions by comparing the intracellular spike events with the extracellularly recorded field and multiple unit activity. Field activity was characterized by either rhythmic theta waves (2–6 Hz) or irregularly occurring sharp wave events (SPW) associated with a fast field oscillation in the pyramidal layer (ripple) and population discharge of a large number of neurons (Ylinen et al., 1995a). Dendritic action potential bursts occurred most consistently during SPWs, although they were occasionally observed during theta activity and between the irregularly occurring SPWs. All recordings illustrated in the present work were obtained during SPW (nontheta) state. The relationship between intracellular events and the extracellularly recorded multiple unit activity (MUA) was quantified by cross-correlating these events. As was the case with somatically recorded action potentials (Ylinen et al., 1995), SPW-associated field ripples and MUA correlated with dendritic bursts of action potentials (Fig. 3E, left inset). A burst of action potentials was defined as repeating spikes at 10–20 msec intervals for these calculations. In each animal with a sufficient number of SPW bursts (>10) for the construction of cross-correlograms (n = 17), the large peaks in the cross-correlograms indicated that intradendritic bursts occurred preferentially during the SPW-associated population events.

Similar to the current injection-induced events, large-amplitude spikes never occurred in isolation but were always part of a dendritic burst. In distal dendrites the amplitude of the small and large spikes could easily be distinguished. To study the role of network activity in the generation of the large-amplitude events, the large-amplitude spikes were cross-correlated with the simultaneously recorded MUA (Fig. 3E, right inset). The peaks in the cross-correlograms indicated that large-amplitude spikes occurred preferentially during the SPW-associated population events (n = 6 distal dendrites). In four cases, spontaneous large-amplitude spikes were present exclusively during SPW bursts.

SPW-associated large, fast spikes, similar to current pulse-
induced events, arose from wide depolarizing potentials. These wide depolarizing potentials probably reflected a combination of synaptically induced postsynaptic potentials and Ca\(^{2+}\) spikes, because their amplitude was often larger than that of the commissurally evoked depolarization (Figs. 3C,E, 4). In addition, the rate of decay of these wide events was faster than their rate of rise, similar to the current step-induced Ca\(^{2+}\) spikes. Putative calcium spikes occurred very rarely (<0.2/min) at the resting potential, but steady depolarization of the dendrite (<10 mV) significantly increased the incidence of putative calcium spikes and revealed them in neurons in which no such events were observed at rest (50–350%; \(t = 2.45; \ p < 0.05; n = 12\)). These observations indicate that at least part of the SPW-associated large depolarizing events reflect Ca\(^{2+}\) spikes. In an attempt to increase the incidence of large-amplitude fast spikes and associated putative Ca\(^{2+}\) spikes, the frequency of sharp wave bursts was increased by reducing inhibition. Systemic injection of the GABA\(_A\) receptor blocker bicuculline (5 mg/kg, i.p.) increased the amplitude of the commissurally evoked population spike and the incidence of SPW bursts (Buzsáki, 1986). Parallel with these network changes, the incidence of large-amplitude spikes also increased (Fig. 5). The distribution of spike amplitude was bimodal, and bicuculline disproportionately increased the number of large-amplitude spikes (Fig. 5, arrows). Similar to the predrug condition, the large-amplitude fast spikes occurred most often in association with SPWs (Fig. 6). The large-amplitude fast spikes, in turn, were associated with putative Ca\(^{2+}\) spikes. Averages triggered by the large-amplitude fast spikes \((n = 3\) rats with bicuculline treatment; \(n = 4\) rats with no drug treatment) revealed that fast spikes preceded the peaks of the putative Ca\(^{2+}\) spikes (Figs. 6D, 7B2). In four control rats, only the vehicle (0.9% NaCl) was injected after 20–60 min of dendrite impalement. This injection procedure had no effect on the electrophysiological properties of the recorded neurons.

**Action potentials may be initiated at multiple sites**

SPW bursts were typically associated with a single spike in the soma, and the number of somatic spikes during bursts never exceeded four (mean ± SD = 1.2 ± 0.21; \(n = 9\), Fig. 7A). In contrast, SPW events often evoked multiple spike bursts with as many as seven fast action potentials in dendrites (mean ± SD = 3.3 ± 0.65; \(n = 16\); \(t = 4.56; \ p < 0.001\)). In addition, comparison of spike autocorrelograms revealed significantly longer refractory periods of somatic spikes (6.9 ± 1.1 msec) than of dendritic action potentials (2.7 ± 0.52 msec; \(t = 2.87; \ p < 0.01\)). In distal dendritic recordings the refractory period could be as short as the action potential when small- and large-amplitude spikes were all considered (Fig. 7A,B). When only large-amplitude spikes recorded in distal dendrites were considered, interspike intervals were longer (Fig. 7B). These observations are compatible with the view that action potentials can emanate from multiple locations.

**DISCUSSION**

Our results demonstrate that (1) the propagation of fast action potentials in dendrites is strongly suppressed in vivo; (2) large-amplitude dendritic fast spikes occur during SPW bursts; (3) large-amplitude dendritic fast spikes are associated with spike bursts and putative Ca\(^{2+}\) spikes; and (4) fast spikes may be initiated in dendrites and may not invade the soma.

**Spike amplitude attenuation in dendrites**

Our in vitro observations of the amplitude decrease of fast spikes with distance from the soma revealed similarities to previous in vitro results (Spruston et al., 1995; Tsubokawa and Ross, 1996; Magee and Johnston, 1997; Jung et al., 1997). However, in in vitro measurements the first spike of the current step-induced train showed little amplitude decrement with distance from the soma, and only subsequent action potentials were attenuated (Callaway and Ross, 1995; Spruston et al., 1995; Tsubokawa and Ross, 1996). This is in contrast to the in vivo situation, because the majority of spontaneous spikes in dendrites were much smaller than in the soma. Thus, the somadendritic amplitude gradient of spontaneous single action potentials in vivo was similar to the later action potentials in evoked trains in vitro. These findings therefore indicate that dendritic propagation of fast action potentials in the intact hippocampus is under a strong tonic suppression. Such suppression of dendritic activity may be brought about by inhibitory interneurons innervating the dendrites of pyramidal cells (Buzsáki et al., 1996; Miles et al., 1996; Tsubokawa and Ross, 1996).

Simultaneous patch-clamp recordings from the soma and different-caliber dendrites showed a step-like reduction in the imaged Ca\(^{2+}\) signal at dendritic branch points (Spruston et al., 1995). Our observations on spontaneously occurring spikes are compatible with the suggestion that dendritic branch points play a critical role in the regulation of action potential back propagation. Although spike amplitudes were somewhat smaller in the middle third of stratum radiatum than in the proximal third, a large step-wise amplitude reduction was evident in the distal third. Most of our anatomically verified impalements were made in the main apical shaft, which in CA1 pyramidal neurons extends up to the distal third of stratum radiatum (Ishizuka et al., 1995). In only one rat was the dendritic spike amplitude <15 mV in the
middle third of stratum radiatum. In this case, the recording was made from a histologically verified second-order thin branch. Because all branches in the outer third of the stratum radiatum and in stratum lacunosum-moleculare are second- or higher-order branches, the small-amplitude spikes recorded from dendrites in this layer were thin dendritic segments. These observations suggest that it is the branching order, and consequently the increased density of A-type \( K^+ \) channels (Hoffman et al., 1997), that may determine the degree of spike attenuation in the dendritic tree rather than the metric distance from the soma.

**Sharp wave bursts facilitate dendritic electrogenesis**

Of all known physiological patterns, SPWs are associated with the most powerful depolarization of pyramidal cells. Although most interneurons also show intense spiking, their population synchrony is severalfold less than that of the pyramidal cells (Csicsvari et al., 1997). Therefore, during SPWs there is a net increase of synaptic excitation. Dendritic recordings revealed three correlated events during SPWs: bursts of spikes, large-amplitude fast spikes, and wide putative \( Ca^{2+} \) spikes. Large-amplitude action potentials typically occurred with small spike bursts, and large fast spikes were associated with putative \( Ca^{2+} \) spikes. In fact, large-amplitude fast spikes always preceded the peak of the \( Ca^{2+} \) spikes, suggesting that the depolarization produced by the enhanced-amplitude spike is a necessary condition for the activation of high-threshold \( Ca^{2+} \) channels. Although large-amplitude fast spikes in distal dendrites occurred almost exclusively during SPWs, only a portion of SPWs (12%) was associated with large amplitude spikes. Spontaneous \( Ca^{2+} \) spikes occurred even more rarely (<0.2/min). The discrepancy between the relative regularity of the population event (SPW) and the rare events in the dendrites may be explained by assuming that coincident presynaptic activity only rarely converges on the recorded dendritic segment. In any case, the amplitude variability of dendritic spikes suggests that action potentials are under the control of local factors. Enhancement of fast spike amplitude may reflect facilitated back-propagation of the action potential from the soma to the recorded dendrite or a locally generated event. Indeed, the average size of action potential decreased progressively toward the distal dendrites. Nevertheless, when convergent network excitation was powerful enough during SPW bursts, large-amplitude spikes were also present in distal dendrites. The enhancement of fast spike amplitude may reflect facilitated back-propagation of the action potential from the soma to the recorded dendrite or a locally generated event. The observation that current-induced intradendritic depolarization enhanced spike amplitude in response to synaptic activation indicates that the magnitude of local membrane depolarization and the speed at which these changes take place are both critical factors.

A general requirement of synaptic plasticity is that afferent activity is present during periods of large postsynaptic depolarization and intradendritic increase of \( Ca^{2+} \) (Bliss and Collingridge, 1993). Recent fluorescent imaging studies indicate the possible involvement of voltage-gated \( Ca^{2+} \) channels in synaptic plasticity (Jaffe et al., 1992; Miyakawa et al., 1992; Regehr and Tank, 1992). Pairing of afferent stimulation with dendritic action potentials can induce a robust \( Ca^{2+} \) influx and long-term modification of the active synapses (Christie et al., 1996; Magee and Johnston 1997; Markram et al., 1997). In light of these experiments, the present observations indicate that in the intact hippocampus these conditions may be present during physiological SPW bursts. During the time window of the SPW, intracellular \( Ca^{2+} \) may be increased by several cooperative mechanisms.
Bursts of Na$^+$-dependent action potentials may lead to Ca$^{2+}$ influx through voltage-gated Ca$^{2+}$ channels (Jaffe et al., 1992; Miyakawa et al., 1992; Christie et al., 1995; Svoboda et al., 1997). Wide Ca$^{2+}$ spikes may further increase intracellular Ca$^{2+}$ levels (Jaffe et al., 1992). The temporal overlap of presynaptic activity and postsynaptic spiking during SPW may result in supralinear summation of Ca$^{2+}$ signals in dendritic spines (Yuste and Denk, 1995; Hoffman et al., 1997; Magee and Johnston, 1997). Finally, the SPW-associated dendritic depolarization and postsynaptic spiking together or separately may facilitate opening of NMDA channels, thus providing another route for Ca$^{2+}$ influx. It has yet to be established whether these various mechanisms are present simultaneously during SPWs, and future research will disclose the mechanism of their cooperativeness.

**Figure 7.** Fast spikes may be initiated at multiple locations. A1, Simultaneous recording of field ripple and intracellular response from soma. Note burst of fast spikes with similar amplitude. A2, Autocorrelogram of fast spikes. Note refractory period of >8 msec. Inset, Averaged somatic spike. B1, Simultaneous recording of field ripple and intracellular response from dendrite. Note the presence of small-amplitude (filled arrow) and large-amplitude (LAS, open arrow) fast spikes. B2, Autocorrelogram of fast large spikes (top) and all spikes (bottom). Note lack of a refractory period when all spikes were included. Inset, Averaged waveforms triggered by large amplitude fast spikes (LAS) and all spikes (all).

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**Fast action potentials may be generated in dendrites**

One of the most intriguing and controversial issues regarding dendritic function is whether action potentials have a fixed site of generation in the axon initial segment or whether they can be initiated at multiple locations (Shepherd and Brayton, 1987; Llinás and Nicholson, 1971; Segev and Rall, 1988; Jaslove, 1992; Softky, 1994; Traub et al., 1994). The initiation of Na$^+$ action potentials in the axon initial segment or the axon and their active back propagation to the dendritic tree have been directly verified under some conditions. The initiation of Na$^+$ action potentials in dendrites is more controversial (Stuart et al., 1997). Dendritically generated spikes in cortical pyramidal cells have been observed by extracellular field recordings and by intracellular recordings with sharp electrodes and patch pipettes (Spencer and Kandel, 1961; Wong et al., 1979; Herreras, 1990; Turner et al., 1991; Wong and Stuart, 1992; Regehr et al., 1993). An important argument against the physiological relevance of these observations is that all experiments used an excessive synchrony of presynaptic fibers induced by electrical stimulation (Mainen et al., 1995; Stuart et al., 1997). Although many of the action potentials recorded in dendrites in vivo likely reflect action potentials initiated in the soma and back-propagating into the dendrites, two observations indicate that action potentials can also be initiated at dendritic locations. First, more spikes were observed during SPW bursts in dendritic recordings than in somatic recordings. The additional dendritic spikes must be generated in the dendrites but must not propagate reliably enough to trigger action potentials at the soma. Second, when small- and large-amplitude spikes were considered, the interspike intervals in dendritic recordings were shorter than expected on the basis of spike refractoriness. The refractory period for dendritically initiated spikes likely extends to some
distance over which the spike propagates actively, but spikes may be generated at other dendritic locations while one dendritic spike initiation zone is refractory. The most parsimonious explanation of our findings is that action potentials emanated from multiple sites. In support of our in vivo observations, simultaneous recordings from the soma and dendrites of CA1 pyramidal cells in vitro indicate that focally triggered spikes in dendrites often fail to invade the soma (N. L. Golding and N. Spruston, personal communication). These findings therefore indicate that the function of action potentials confined to the dendrites is fundamentally different from somatic action potentials that signal via the axon. One of these functions may be to trigger local Ca$^{2+}$ spikes and modify synaptic strength.

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