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Pattern and inhibition-dependent invasion of pyramidal cell dendrites by fast spikes in the hippocampus *in vivo*

(dendritic spikes/plasticity/sharp waves/behavior/interneurons)

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ABSTRACT **The invasion of sodium spikes from the soma into dendrites was studied in hippocampal pyramidal cells by simultaneous extracellular and intracellular recordings in anesthetized rats and by simultaneous extracellular recordings of the somatic and dendritic potentials in freely behaving animals. During complex-spike patterns, recorded in the immobile or sleeping animal, dendritic invasion of successive spikes was substantially attenuated. Complex-spike bursts occurred in association with population discharge of CA3-CA1 pyramidal cells (sharp wave field events). Synaptic inhibition reduced the amplitude of sodium spikes in the dendrites and prevented the occurrence of calcium spikes. These findings indicate that (*i*) the voltage-dependent calcium influx into the dendrites is under the control of inhibitory neurons and (*ii*) the temporal coincidence of synaptic depolarization and activation of voltage-dependent calcium conductances by the backpropagating spikes during sharp wave bursts may be critical for synaptic plasticity in the intact hippocampus.**

Traditional models of neuronal function state that fast (sodium) spikes are generated in the axon hillock or axon initial segment and passively propagate back to the dendrites, although it has also been suggested that dendrites play an active role in spike propagation (1–5). Recent *in vitro* work provides evidence that the dendritic membrane can exhibit electroresponsive properties and actively support propagation of action potentials in dendritic compartments (6–10). Specifically, simultaneous recordings from the soma and dendrites of neocortical and hippocampal pyramidal cells *in vitro* suggest that sodium spikes, initiated in the axon initial segment, actively backpropagate into the distal dendrites (8, 9). Two aspects of the backpropagating action potentials make these findings particularly relevant to the operations of hippocampal networks. First, calcium influx into the cell depends on the number and frequency of sodium spikes successfully invading the dendrites (9, 11). Consequently, repetitive fast spikes in individual dendrites may be involved in synaptic plasticity, a phenomenon dependent on calcium influx (12). Second, success or failure of dendritic invasion in a spike series also depends on the level of membrane polarization (9). These observations suggest that state-dependent activity of layer-specific inhibitory interneurons (13–15) in the behaving animal may selectively modulate the effectiveness of action potential backpropagation and hence regulate synaptic plasticity (16, 17). To address these issues, somatic and dendritic potentials, generated by hippocampal pyramidal neurons, were monitored simultaneously in both anesthetized and freely behaving rats.

MATERIALS AND METHODS

Experiments Under Anesthesia. Twenty-six rats (250–350 g) were anesthetized with urethane (1.3–1.5 g/kg) and placed in a stereotaxic apparatus. The scalp was removed and a small bone window (2.0 mm) was drilled above the hippocampus for extra- and intracellular recordings. Pairs of stimulating electrodes were inserted into the left and right fimbria-fornix to stimulate the commissural inputs to the CA1 region (15). Extracellular recording electrodes (three 20-μm insulated tungsten wires) were inserted into the CA1 pyramidal layer, stratum radiatum, and the dentate hilus (15). Micropipettes for intracellular recording from the soma and dendrites of pyramidal cells were filled with 1 M potassium acetate in 50 mM Tris buffer, also containing 3% biocytin for intracellular labeling. *In vivo* electrode impedances varied from 60 to 100 MΩ. The bridge was balanced and capacitance compensation was used in all experiments. The intracellular DC signal from the amplifier (Axoclamp-2B, Axon Instruments, Foster City, CA) and the extracellular field activity (1–5 kHz) were digitized at 20 kHz with 12-bit precision (18). After the physiological data had been collected, biocytin was injected through the bridge circuit (15, 18). The animals were perfused and the brains were processed for biocytin labeling (15).

Chronic Preparations. Ten male rats (300–450 g) were used in the chronic experiments. Pairs of stimulating electrodes were placed in the angular bundle and into the ventral hippocampal commissure to stimulate hippocampal afferents (18). For simultaneous recording of the action potential generated field, silicon probes micromachined with thin-film technology (18) were used. Each of the six shanks had four recording sites (9×9 μm^2 platinum-plated pads) that were spaced 25 μm apart along the length of the shank. The shanks were 300 μm apart. The thickness of the silicon shank was 15 μm throughout. The silicon probes, attached to a movable headstage, were inserted into the neocortex during surgery. After recovery of the animal, the tips were gradually lowered into the CA1 pyramidal layer for unit isolation. Units that displayed spontaneous complex-spike burst patterns were classified as pyramidal cells (19, 20). Physiological data were recorded wide-band (1–5 kHz for units and field) and sampled at 20 kHz/channel with 12-bit precision. Spikes were extracted by a spike detection algorithm. Spike waveforms were reconstructed and represented by 240 points with floating-point precision and a three-dimensional cluster cutting was used to separate spikes. For the present work, only the largest amplitude units with very clear cluster boundaries were used. Complex-spikes (spike intervals of 2–7 msec) were extracted from the isolated clusters. Following completion of the experiments, the rats were deeply anesthetized and perfused with a fixative. The brain sections were stained by the Gallyas method, and the exact location of the electrodes was determined.

RESULTS

Pattern-Dependent Invasion of Dendrites. The first set of experiments aimed to replicate the *in vitro* observations of

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Spruston *et al.* (9) *in vivo* by using a combination of intrasomatic and extracellular recording electrodes. These investigators observed that action potentials backpropagated into the dendrites in an activity-dependent manner; those occurring early in a train propagated actively, whereas those occurring later failed to actively invade the dendrites. The rationale of our *in vivo* experiment was based on the observation that extracellularly recorded action potentials can be recorded as far as 150 μm from the soma and on the assumption that the extracellularly recorded field is an integrated signal deriving from both soma and dendrites (21). We hypothesized that if dendritic backpropagation of the action potential is activity-dependent, then the amplitude of the extracellular signal should show an activity-dependent decrease. Fig. 1 illustrates one of the two successful experiments with simultaneous extracellular and intracellular recordings. Somatic current injection resulted in a train of action potentials that was faithfully recorded by both the intracellular and extracellular electrodes. The amplitude of successive action potentials, recorded by the intrasomatic electrode, showed very little attenuation (<2%). In contrast, the extracellular action potentials showed a significant ($P < 0.001$; F[2/168] = 19.4; ANOVA) amplitude decrement (Fig. 1). The differential amplitude-attenuation of the extracellular spikes, relative to the intrasomatically recorded action potentials, is further supported by the significant interaction between spike number and intracellular/extracellular categories ($P < 0.001$; F[2/68] = 10.4). The magnitude of the extracellularly measured spike attenuation was less than that reported using intracellular recording *in vitro*. However, in those experiments measurements were made from dendrites up to 400 μm from the soma, whereas in our case the extracellular wire electrode likely integrated both the somatic signal and the currents generated by the proximal dendrites.

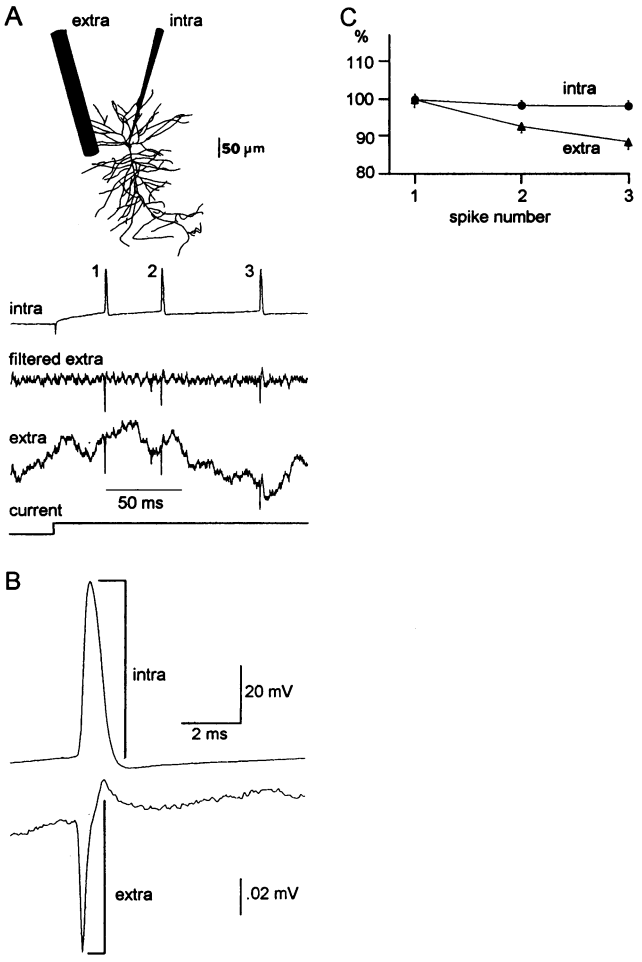

FIG. 1. Amplitude variation of extracellularly recorded action potentials. (*A*) Scheme of simultaneous extracellular and intracellular recordings from the same CA1 pyramidal neuron. The cell was injected with biocytin and reconstructed with the aid of a drawing tube. Depolarizing current to the soma (+0.2 nA) evoked sodium spikes that were also detected by the extracellular recording electrode (0.5–5 kHz, filtered extra; wide-band, 1–5 kHz, extra). (*B*) Averaged intracellular and extracellular action potentials from the same pyramidal cell (n = 80; first spikes after depolarization). Labels to the right of the vertical lines indicate the method of amplitude measurement. (*C*) Amplitude decrement of successive fast spikes recorded intrasomatically and extracellularly (percent of first spike). Note that the amplitude of the extracellularly recorded spikes was attenuated, whereas intrasomatic action potentials were only slightly affected. Vertical bars: SEM.

The next set of experiments addressed the relevance of spike backpropagation failures in the drug-free animal. In extracellular recordings, repetitive spikes with decrementing amplitude occur spontaneously during consummatory behaviors and slow-wave sleep in hippocampal pyramidal cells and are termed "complex-spike" bursts (20). We hypothesized that complex-spikes in the extracellular field emerge because the extracellular electrode integrates electrical activity over a large segment of the somadendritic surface and because spikes occurring in fast succession fail to invade the dendrites equally.

Linear arrays of silicon probes with recording sites spaced at 25-μm intervals were closely positioned to CA1 or CA3 pyramidal neurons, so that sodium spikes could be recorded from the same cell(s) at four recording sites. Based on the depth profiles of the population spikes (1, 5), we assumed that recording sites with the largest amplitude action potentials corresponded to perisomatic recording, whereas other sites preferentially recorded action potentials from the basal or apical dendrites (Fig. 2). Virtually all complex-spike bursts occurred during sharp wave events (18) while the rat was sitting motionless or in slow wave sleep.

The propagation velocity of action potentials along the somadendritic membrane ranged 0.5 to 2 m/sec in different cells (0.82 ± 0.12; n = 6 CA1, n = 5 CA3 neurons; Fig. 2). No differences were observed between CA1 and CA3 pyramidal cells. These values are 2 to 8 times faster than those measured in hippocampal pyramidal cells at room temperature *in vitro* (9) using patch-clamp recording. The discrepancy may be explained by the differences in temperature or by the different recording conditions. Our methods allowed measurements to be made only from the first- and perhaps second-order dendrites, whereas patch-clamp measurements were also available from thin dendrites several hundred micrometers from the parental soma.

Consistent with earlier observations (20), the amplitude decrement of spikes during a complex-spike burst was frequency-dependent; spikes occurring at shorter intervals were attenuated more than spikes with longer interspike intervals. However, the degree of spike amplitude attenuation also depended on the recording site. At recording sites with the largest amplitude spikes, no or only a moderate amplitude decrement was observed within a complex-spike burst (Fig. 3*A*, site 4). At the same time, the amplitude attenuation at more distal recording sites (presumed dendritic locations) was often substantial (Fig. 3*A*, sites 2 and 3). Late occurring action potentials at dendritic recording sites were sometimes buried in the background noise. Due to these constraints, group analysis of complex-spike bursts was confined to the first three consecutive spikes of the largest amplitude units (>0.5 mV; n = 7 cells). The group averages (Fig. 3*B*) confirmed that the amplitude of the second and third spikes was significantly more attenuated at the dendritic (distal) than at the assumed somatic locations ($P < 0.05$; ANOVA). A parsimonious interpretation of this observation is that spikes successfully invading the dendrites by an active mechanism generated larger extracel-

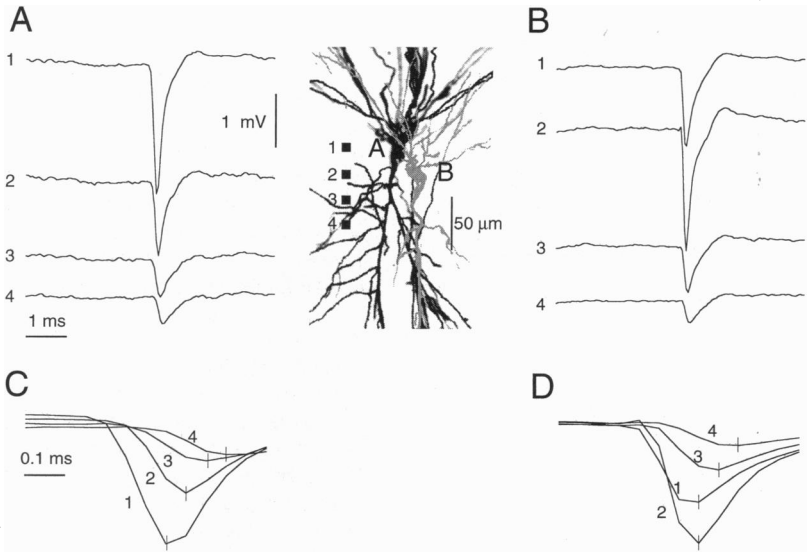

FIG. 2. Propagation speed of sodium spike in CA1 pyramidal cells *in vivo*. Averaged action potentials (n = 256) from the four recording electrodes from cells A and B are shown at left and right, respectively. Filter settings: 1–5 kHz; sampling rate: 20 kHz. (*Inset*) Hypothesized relationship between recording sites (■; 25-μm intersite intervals) and spatial position of two simultaneously recorded cells, drawn from two filled cells of the intracellular experiments. (*C* and *D*) Details of the action potential peaks shown in *A* and *B*, respectively. Note that in *A* spikes occur first at the site with the largest amplitude action potential (site 1; putative somatic region of the neuron). Such a relationship between spike amplitude and peak latency was typical in the majority of neurons. In neuron B (a rare case), the spike occurred simultaneously or earlier at site 1 than at the site with the largest amplitude action potential (site 2). Spike at site 1 may reflect sodium spike initiation at the axon initial segment.

lular currents than spikes that failed to regenerate fully in the dendrites.

Interneuron Modulation of Dendritic Spike Invasion. These experiments examined whether synaptic inhibition may interfere with spike backpropagation in the dendrites. Stable intradendritic impalements were made 100–200 μm below the pyramidal cell layer (n = 8; neurons held longer than 30 min). Inhibitory interneurons were activated by stimulation of the commissural input (15). Weak stimuli elicited only inhibitory postsynaptic potentials in the pyramidal neurons. At higher intensities, population spikes were recorded with the extracellular electrode placed in the pyramidal layer and the discharging CA1 pyramidal cells recruited further inhibition by their recurrent interneurons (13–16). Dendritic current injection elicited fast sodium spikes and one or two larger amplitude, wide (30–60 msec) spikes (Fig. 4*A*). We regarded these wide spikes as putative Ca^{2+} spikes (4). Low intensity commissural volleys could delay or abolish the Ca^{2+} spike altogether (Fig. 4*A b* and *c*). If the commissural volley arrived after the emergence of the Ca^{2+} spike, it was aborted (Fig. 4*A d*). When the intensity of commissural stimulation was increased further, the amplitude of late-occurring fast spikes decreased considerably (Fig. 4*A e*). The amplitude decrement was largest for the first spike and the time course of the amplitude recovery paralleled the decay of the inhibitory postsynaptic potential, evoked by the commissural stimulus. These observations were consistent in all eight cells with intradendritic recordings. The results indicate that activation of interneurons with synapses on the CA1 pyramidal cell dendrites (14, 15) can attenuate the active regeneration of the backpropagating sodium spikes. In the absence of the regenerative process, the passively propagating sodium spikes may not invade more distal dendrites and fail to induce calcium-dependent spikes.

Intradendritic recordings also confirmed activity-dependent attenuation of spike amplitude. When a spontaneously occurring spike preceded the synaptically evoked action potential at a short interval, the amplitude of the evoked spike decreased (Fig. 4*B*). Such amplitude attenuation was observed in four out

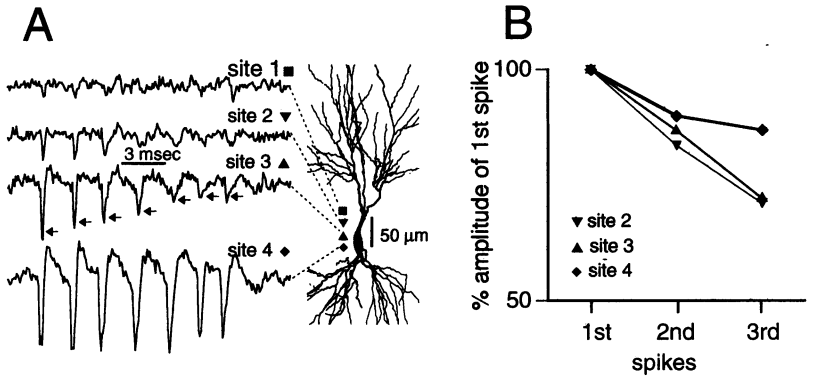

FIG. 3. Attenuation of sodium spike propagation in pyramidal cell dendrites during complex-spike bursts. (*A*) Complex-spike burst of seven action potentials at the soma (filter, 0.1–5 kHz; sampling rate, 20 kHz). (*Right*) Hypothesized relationship between recording sites (25 μm intersite intervals) and the recorded CA3 pyramidal cell. Note amplitude decrement of successive actions potentials at recording site 3, 2, and 1 (arrows in site 3). (*B*) Average amplitude decrease of action potentials during complex-spike bursts in a group of seven CA3 pyramidal cells, each cell representing 50–100 complex-spike bursts. Note that spike amplitude attenuation is larger at the more distal (dendritic) locations (sites 2 and 3) than at the soma (site 4).

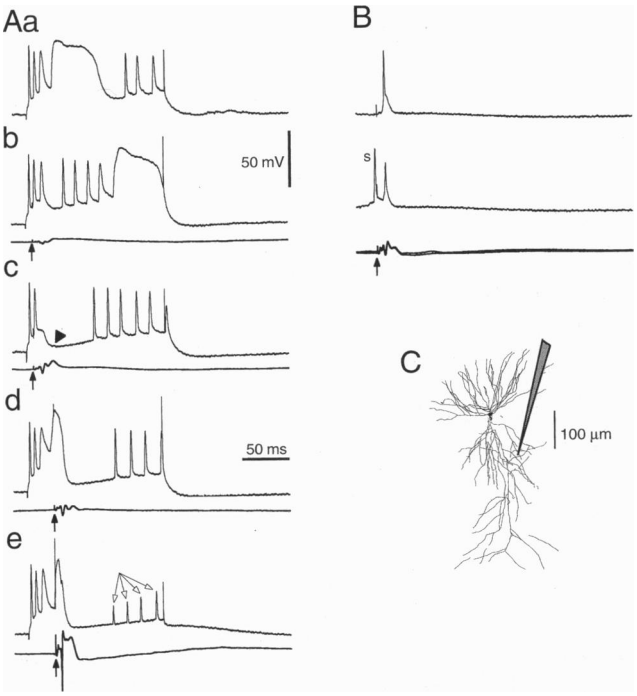

FIG. 4. Inhibition-induced changes in the active dendritic propagation of fast spikes. (*A*) Dendritic recording from a CA1 pyramidal cell. Approximate depth of dendritic penetration is shown in *C*. (*A a*) Intradendritic depolarization (0.4 nA) evoked fast spikes and a slow spike. (*b–e*) Commissural stimulation (short arrows) paired with dendritic depolarization (0.4 nA). Bottom traces, extracellular records from the pyramidal layer. Weak commissural stimulation delayed (*b*, 30 μA), abolished (*c*, 50 μA) or aborted the slow spike (*d*, 50 μA). When the intensity of commissural stimulation was further increased (*c*, 450 μA) the amplitude of the fast spikes substantially decreased (long arrows). (*c*) ▲, inhibitory postsynaptic potential evoked by the commissural stimulus. (*B*) Synaptically evoked fast spike (100 μA) decreased in amplitude when preceded by a spontaneously occurring spike(s). (*C*) After the recordings, the cell was injected with biocytin and reconstructed with the aid of a drawing tube. The site of dendritic penetration was estimated from the distance between the electrode tip and the pyramidal layer.

four of dendritic recordings. No such amplitude decrement of the action potential was observed in pyramidal cells recorded from the soma (n = 8 cells; data not shown).

DISCUSSION

These experiments in anesthetized and behaving rats indicate that (*i*) dendritic spike propagation is modified during behaviorally relevant physiological patterns, such as complex-spike bursts and (*ii*) spike invasion of the dendrites is controlled by inhibitory neurons with dendritic targets. Since dendritic action potentials trigger voltage-gated entry of calcium (9–11), these *in vivo* findings suggest that behavior-dependent activity of interneurons may play a role in cellular plasticity.

At least two factors appear critical in influencing the efficacy of active spike travel in pyramidal cell dendrites: spike frequency and the momentary polarization level of the dendritic segment (9, 22). Bursts of spikes with short interspike intervals and decrementing amplitude ("complex-spike" events; ref. 20) can be recorded extracellularly in association with large field sharp waves during consummatory behaviors and slow wave sleep (18, 23). The mechanisms of complex-spike burst generation are not known, although it has been tacitly assumed that they reflect "intrinsic" regenerative properties of pyramidal cells (19, 20, 24). We hypothesize that the spike order-dependent amplitude attenuation of the extracellularly recorded spikes reflects a progressive attenuation of somadendritic propagation of the action potentials.

Besides the intrinsic, activity-dependent modulation, dendritic spike propagation is also influenced by network effects. Intradendritic recordings revealed an amplitude decrement of the action potentials when sufficient inhibition was recruited by commissural stimulation. Dendritic hyperpolarization or shunting of the membrane resistance (25) may have been mediated by either bistratified or trilaminar interneurons, since these neurons densely innervate the dendritic domains of pyramidal cells and can be activated by commissural afferents (14, 15). Inhibition of dendritic action potential propagation may have important implications for synaptic plasticity. Selective and restricted activation of inhibitory interneurons with highly specific and stratified innervation of pyramidal cells (13–15) may spatially restrict the somatofugal propagation of fast action potentials and voltage-activated influx of calcium (9, 11, 16).

An important aspect of the *in vivo* observations is that complex-spike events of individual pyramidal neurons occur at times of highest population synchrony in the behaving animal, i.e., during sharp wave bursts events (18, 23). During the sharp wave event (50–100 msec), a large number of pyramidal cells and interneurons discharge in concert, and many of them emit complex-spike bursts. Pyramidal cells are strongly depolarized during these population bursts, due to the simultaneous activity of their presynaptic partners (18), and therefore may allow calcium entry by the activation of *N*-methyl-D-aspartate receptors. Another route of calcium entry in bursting neurons is through voltage-gated calcium channels (9, 11, 26). Therefore, it is reasonable to assume that sodium spike-triggered and synaptically mediated calcium influx mechanisms (27) interact with each other during the time window of the sharp wave-associated population bursts. The importance of such an interaction for synaptic plasticity is underlined by the supralinear calcium accumulations in spines of pyramidal cells when synaptic and spike activation occur simultaneously (26). A general requirement of synaptic plasticity is that afferent activity is present during periods of large postsynaptic depolarization and dendritic calcium influx (12). The temporal coincidence of synaptic depolarization and activation of voltage-dependent calcium conductances by the backpropagating spikes *in vivo* may therefore restrict synaptic modification to active synapses only. In addition, activity-dependent filtering and inhibition-induced attenuation of dendritic backpropagation of sodium spikes may differentially affect the laminarly segregated associational and entorhinal inputs by limiting potentiation to synapses terminating on more proximal parts of the dendrites (i.e., the associational inputs).

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