Synaptically Evoked Dendritic Action Potentials in Rat Neocortical Pyramidal Neurons

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Schwindt, Peter C. and Wayne E. Crill. Synaptically evoked dendritic action potentials in rat neocortical pyramidal neurons. J. Neurophysiol. 79: 2432–2446, 1998. In a previous study iontophoresis of glutamate on the apical dendrite of layer 5 pyramidal neurons from rat neocortex was used to identify sites at which dendritic depolarization evoked small, prolonged Ca2+ spikes and/or low-threshold Na+ spikes recorded by an intracellular microelectrode in the soma. These spikes were identified as originating in the dendrite. Here we evolve similar dendritic responses by electrical stimulation of presynaptic elements near the tip of the iontophoretic electrode with the use of a second extracellular electrode. In 9 of 12 recorded cells, electrically evoked excitatory postsynaptic potentials (EPSPs) above a minimum size triggered all-or-none postsynaptic responses similar to those evoked by dendritic glutamate iontophoresis at the same site. Both the synaptically evoked and the iontophoretically evoked depolarizations were abolished reversibly by blockade of glutamate receptors. In all recorded cells, the combination of iontophoresis and an EPSP, each of which was subthreshold for the dendritic spike when given alone, evoked a dendritic spike similar to that evoked by a sufficiently large iontophoresis. In one cell tested, dendritic spikes could be evoked by the summation of two independent subthreshold EPSPs evoked by stimulation at two different locations. We conclude that the dendritic spikes are not unique to the use of glutamate iontophoresis because similar spikes can be evoked by EPSPs. We discuss the implications of these results for synaptic integration and for the interpretation of recorded synaptic potentials.

INTRODUCTION

In a previous study (Schwindt and Crill 1997) we found that the iontophoresis of glutamate on the apical dendrite of a layer 5 pyramidal neuron could evoke action potentials that arose in the dendrite, probably at or near the iontophoretic site. Two types of dendritic action potentials were observed. One was small and prolonged (as recorded in the soma) and appeared to be a Ca2+ served. One was small and prolonged (as recorded in the depolarization of the same dendritic region by an EPSP that arose in the dendrite, probably at or near the iontophoretic electrode, might synapse on the same dendritic area that gave rise to the iontophoretically evoked spike, and there-

METHODS

Most methods were similar to those described previously (Schwindt and Crill 1995–1997). Sprague-Dawley rats of either sex (28–35 d postnatal) were anesthetized with ketamine (150 mg/kg) and xylazine (10 mg/kg) and killed by carotid section. A coronal section of cortex 0- to 3-mm posterior to the bregma was isolated and slices 350–μm thick were prepared and maintained as described. Recorded cells lay 1.11–1.26 mm below the pial surface and 2.74–2.96 mm from midline, corresponding to layer 5 of areas FL and HL of sensorimotor cortex (Zilles and Wree 1985).
Recordings were made in a chamber with the slice submerged and maintained at 33 ± 0.2°C. Slices were perfused with a physiological saline consisting of (in mM) 130 NaCl, 3 KCl, 2 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, and 10 dextrose saturated with 95% O₂–5% CO₂, pH 7.4. In some experiments d-2-amino-5-phosphonopentanoic acid (APV; 50 μM) and the disodium salt of 6-cyano-7-nitroquinazoline-2,3-dione (CNQX; 50 μM), both from Tocris Cookson (Ballwin, MO), were added to this saline.

Cells were impaled with sharp microelectrodes made from standard borosilicate tubing, 1.0 mm OD containing 2.5 M KCl (DC resistance 30–40 MΩ). An Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) was used to record membrane potential and to inject current intracellularly in the bridge mode.

For dendritic stimulation we employed a double-barrel piggyback extracellular electrode (PB150F, World Precision Instruments, Sarasota, FL). The thinner of the two barrels was broken to a tip diameter of ~2 μm and filled with 0.5 M Na glutamate buffered to pH 7.4 with 30 mM N-2-hydroxymethyl piperazine-N’-2-ethanesulfonic acid (HEPES). The second current-clamp amplifier and headstage of the same Axoclamp-2A was used to pass a constant current through this iontophoretic barrel. Negative iontophoretic currents of 20–100 nA were employed from a +5 nA holding current. Positive iontophoretic currents never produced a postsynaptic response.

The thicker of the two piggyback barrels was broken to a tip diameter of 5–7 μm and filled with Ringer solution buffered to pH 7.4 with HEPES. This barrel was used for focal monopolar electrical stimulation. One lead of an optically isolated, constant-current stimulation unit was inserted into this barrel, and the other lead was attached to an Ag-AgCl pellet in the bath. This pellet was separate from that providing the bath reference potential for the Axoclamp amplifier. In some experiments extracellular electrical stimulation at the pial surface also was employed with a separate constant-current stimulus isolation unit with a bipolar electrode consisting of a twisted pair of 0.1-mm-OD stainless steel wires insulated except for their tips. This electrode was positioned 200–500 μm from the intersection of the apical dendrite of the recorded cell with the pial surface as estimated from the position of the recording and iontophoretic electrodes. Pulse durations of 0.1 ms were used for electrical stimulation.

The piggyback electrode was positioned by using a separate micromanipulator, and a dendritic site was found near a line extending from the recording electrode normal to the pial surface. Postsynaptic responses, evoked by iontophoresis of 100–200 ms repeated each 15–20 s, were stable and reproducible. Electrical stimulation used to evoke EPSPs was repeated every 2–5 s. The distance between recording and stimulating electrodes was measured at the slice surface with the use of a calibrated eyepiece on a dissecting microscope.

Membrane potential, injected current, and iontophoretic current were monitored, amplified, filtered at 10 kHz, and recorded on a multichannel video cassette recorder with pulse code modulation (Neuro-Data, New York, NY). Resting potential was taken as the difference between the intracellular and extracellular DC potentials recorded on a chart recorder. In some experiments the time-derivative of recorded membrane potential was computed by leading membrane potential into an electronic differentiator whose voltage output was proportional to dV/dt. Recorded data were played back into a storage oscilloscope for photography or digitized for further analysis by computer.

RESULTS

Cell properties

Recordings were made from 12 cells that had electrical properties similar to those described in recent investigations from our laboratory (Schwindt and Crill 1995–1997). Resting potential averaged −75 mV (range: −70 to −80 mV). Input resistance measured by 1-s duration hyperpolarizing injected current pulses averaged 23.8 MΩ. Two cells had substantially higher input resistance (45.0 and 38.7 MΩ) than the other ten (14.0–29.2 MΩ). In response to 1-s duration depolarizing injected current pulses these two high-resistance cells displayed a slowly adapting repetitive firing pattern, whereas the low-resistance cells were fast-adapting (n = 5), high-threshold bursters (n = 3), or low-threshold bursters (n = 2) as described in Schwindt et al. (1997). Thus the electrical properties of the cells recorded in this study spanned the range that we observed previously.

Dendritic spikes evoked by glutamate iontophoresis

Using glutamate iontophoresis at sites on the apical dendrite, we were able to evoke dendritic spikes of the type described previously (Schwindt and Crill 1997) in every cell. Thus our earlier study appears to have underestimated the percentage of cells that can generate a dendritic spike. In that study we used a 1-s iontophoresis whose strength was restricted to cause a subthreshold depolarization or low-rate repetitive firing, and we noticed that a dendritic spike was evoked at the onset of the 1-s iontophoresis (or evoked in isolation by using a short iontophoresis of the same strength) in 71% of the cells. In the present study we used only short (100–200 ms) iontophoresis, and we found that a dendritic spike could be evoked in 100% of the recorded cells if we simply increased the iontophoretic strength to a level that presumably depolarized an adequate length of the dendrite by an adequate amount. In addition, we were able to evoke similar spikes by electrically evoked EPSPs.

Figure 1A shows the response of one cell to a iontophoresis of 200-ms duration that evoked both types of dendritic spikes observed previously (Schwindt and Crill 1997). At the iontophoretic strength employed in Fig. 1A, a smooth subthreshold response was obtained on some sweeps (trace 1), a large, fast spike with a low apparent threshold was obtained on other sweeps (trace 2), and this alternated randomly with a small, prolonged spike (trace 3). The small spike (trace 3) is similar in amplitude and duration to those identified as Ca²⁺ spikes previously because they were abolished by blockade of voltage-gated Ca²⁺ channels but not by blockade of voltage-gated Na⁺ channels with tetrodotoxin (TTX) nor by blockade of N-methyl-D-aspartate (NMDA) receptors (Schwindt and Crill 1997). The large, fast, low-threshold spikes were abolished by TTX (Schwindt and Crill 1997). On the basis of these previous results, we will refer to the small, slow spikes as Ca²⁺ spikes and the large, fast, low-threshold spikes as Na⁺ spikes.

These two types of spikes are shown at a faster sweep speed in Fig. 1B, which more clearly illustrates their low apparent threshold. They rise abruptly from the underlying smooth depolarization, which is more than a few millivolts positive to resting potential. The low apparent threshold of the iontophoretically evoked Na⁺ spike contrasts with the threshold of the Na⁺ spike evoked by intrasomatic current injection (cf. Fig. 1C). Figure 1D1 shows one of the Ca²⁺ spikes at a fast sweep speed and the smooth, subthreshold depolarization observed when the Ca²⁺ spike failed. The
digital subtraction of these two traces reveals the Ca$^{2+}$ spike waveform (Fig. 1D2). The afterhyperpolarization seen in Fig. 1, D1 and D2, was a consistent feature of the Ca$^{2+}$ spikes.

We observed previously that the low-threshold Na$^{+}$ spike often was associated with an underlying Ca$^{2+}$ spike (Schwindt and Crill 1997). This association was confirmed in the present study and was, in fact, clearer and more consistent because we specifically searched for an underlying Ca$^{2+}$ spike when we observed a low-threshold Na$^{+}$ spike. When the low-threshold Na$^{+}$ spike was evoked, the associated Ca$^{2+}$ spike often was not apparent (Fig. 1B, trace 2). In most of the six cells that displayed a low-threshold Na$^{+}$ spike in this study, the associated Ca$^{2+}$ spike was revealed only during strong hyperpolarization of the soma (e.g., Fig. 8C) or when the low-threshold Na$^{+}$ spike was occluded by a somatic Na$^{+}$ spike (e.g., Fig. 3B, trace 2).

We examined the response to iontophoresis at two to three distances from the soma in eight of the cells, and we found that the iontophoresically evoked response tended to change character with distance from the soma. In the cell of Fig. 1, the dendritic spike was seen only during iontophoresis 463 $\mu$m from the soma. Iontophoresis at 315 $\mu$m evoked only a smooth, graded depolarization that evoked a Na$^{+}$ spike with the same apparent threshold as that evoked by intrasomatic current injection (not shown). Another cell displayed only a small Ca$^{2+}$ spike at both 370 and 444 $\mu$m from the soma. In the other six cells, low-threshold Na$^{+}$ spikes (and underlying Ca$^{2+}$ spikes) were evoked when the distance to the soma was <400 $\mu$m. At larger distances only the small Ca$^{2+}$ spike could be evoked. These results raise the possibility that the predominant type of dendritic spike in the remote dendrites (≥400 $\mu$m from the soma) is a local (nonactively propagated) Ca$^{2+}$ spike. Dendritic Na$^{+}$ spikes, at least those that propagate actively to the soma, may be triggered more commonly only in the proximal half of the apical dendrite.

In our previous study (Schwindt and Crill 1997) we observed that low-threshold Na$^{+}$ spikes could be evoked only at the beginning of a long-lasting iontophoresis. Na$^{+}$ spikes evoked later during the iontophoresis had a higher threshold, equivalent to that of Na$^{+}$ spikes evoked by somatic depolarization (cf. Fig. 2, A and B). A possible explanation for this observation is that adequately sustained depolarization inactivates the dendritic Na$^{+}$ spike mechanism. This idea was tested by applying a constant short iontophoresis that evoked a low-threshold Na$^{+}$ spike while depolarizing the soma to different steady potentials by DC current injection. Depolarizing the soma also depolarizes some portion of the dendrite, and somatic depolarization might be effective if the low-threshold Na$^{+}$ spike is usually initiated in the prox-
Dendritic spikes evoked by EPSPs

After examining the response of a cell to iontophoresis, we examined the effect of extracellular electrical stimulation through the other barrel of the piggyback electrode (see METHODS). Adequate cathodal stimulation evoked an EPSP in all recorded cells and the stimulus was increased until a spike was evoked. Anodal stimulation at the same strength never evoked a response. In seven of nine cells in which iontophoresis evoked a low-threshold Na⁺ spike, the EPSP also evoked a low-threshold Na⁺ spike. Figure 3 shows an example from the same cell as Fig. 1. Figure 3A shows the low-threshold Na⁺ spike that was evoked all-or-none by local extracellular electrical stimulation. (The decay of the large stimulus artifact in Fig. 3A obscures the rising phase of the underlying EPSP.) Voltage gain and resting potential are identical in Figs. 3A and 1. By comparing the depolarization from resting potential needed to evoke a spike in the two figures, it is apparent that the threshold of the spike of Fig. 3A is similar to that evoked by iontophoresis (cf. Fig. 1B) and far below that evoked by somatic current injection (cf. Fig. 1C).

Preceding an iontophorically evoked spike with an EPSP-evoked spike prevented the initiation of the former and vice-versa. The iontophoresis in Fig. 3B evoked a low-threshold Na⁺ spike followed by a slow depolarization (trace 1). When the iontophorically evoked Na⁺ spike was preceded by the synaptically evoked Na⁺ spike (trace 2, left), the iontophorically evoked Na⁺ spike failed and a delayed Ca²⁺ spike appeared instead (trace 2, right). The records shown are representative of each of 10 repetitions of this stimulus pairing. If anodal electrical stimulation was applied, or if the electrical stimulus was reduced to evoke only a subthreshold EPSP, the iontophorically evoked spike was unaffected (data not shown). In Fig. 3, C and D, the same extracellular stimulus was applied after the iontophorically evoked response, which alternated randomly between a Ca²⁺ spike (Fig. 3C) and a low-threshold Na⁺ spike (Fig. 3D) during different sweeps. The asterisks in Fig. 3, C and D, mark the extracellular stimulus artifacts. When applied after either type of iontophorically evoked spike, the extracellular stimulus evoked only an EPSP. That is, the EPSP-evoked low-threshold Na⁺ spike was occluded. The time interval over which the prior initiation of one type of spike (e.g., EPSP-evoked) prevents the initiation of the other type (e.g., iontophorically evoked) lasted tens of milliseconds, far longer than expected for recovery from Na⁺ inactivation. We call this more complex spike interaction, resulting from unknown mechanisms, “occlusion” to distinguish it from the normal spike refractory mechanism. The traces shown in Fig. 3, C and D, also are representative of results obtained during each of 10 repetitions of the stimulus pairings. These results indicate that both the iontophorically evoked and the EPSP-evoked spikes arose in the same dendritic region.

From the records of Fig. 1D it may be appreciated that the iontophorically evoked Ca²⁺ spike can resemble an EPSP in terms of its general shape, amplitude, and duration. Consequently, it would be difficult or impossible to distinguish a Ca²⁺ spike from a component of the EPSP merely by inspection of the evoked potential. To investigate this question, we looked for an all-or-none fluctuation of an EPSP-evoked potential that resembled the Ca²⁺ spike evoked by iontophoresis at the same site. Figure 4, taken from the same cell as Figs. 1 and 3, presents evidence that an EPSP can trigger a Ca²⁺ spike. Because the EPSP evoked at resting potential resulted in a low-threshold Na⁺ spike in...
this cell (see Fig. 3A), it was necessary to evoke the EPSP during a hyperpolarization of the soma to prevent Na⁺ spike initiation while searching for a possible underlying Ca²⁺ spike. The superimposed records in Fig. 4A show the experimental paradigm employed. One sweep without stimuli is shown in Fig. 4A to indicate the baseline membrane potential during the hyperpolarization. Two cathodal electrical stimuli were applied during this hyperpolarization (arrows labeled 1 point to the corresponding stimulus artifacts). Two consecutive sweeps of the response to these cathodal stimulus pairs are superimposed in Fig. 4A to show the different responses evoked by the same stimuli. The second of the two stimuli evoked a larger response during one sweep (trace b) and a smaller response during the other sweep (trace a). Also shown is the absence of a response to an anodal stimulus pair of the same strength (arrows labeled 2 point to corresponding stimulus artifacts).

Figure 4B shows (at faster sweep speed) 10 superimposed consecutive responses to the cathodal stimulus pairs applied during the hyperpolarization of Fig. 4A. It is apparent that the second, larger, subthreshold response (trace b) occurs in an all-or-none manner at this stimulus strength. In fact, the stimulus strength was selected to evoke the larger second response in an all-or-none manner. At lower strengths, both the first and second responses were graded with stimulus strength. A larger stimulus evoked only the larger second response and a low-threshold Na⁺ spike. The records of Fig. 4B also show that the larger second response (trace b) occasionally evoked Na⁺ spikes (trace c). By comparing the threshold of these Na⁺ spikes above the baseline membrane potential in Fig. 4B with the baseline membrane potential in Fig. 4A (taken at the same gain), it can be appreciated that the apparent threshold of these action potentials would be just positive to resting potential. That is, the larger second response was capable of triggering low-threshold Na⁺ spikes.

Figure 4C1 shows superimposed responses to eight stimulus pairs during which the larger second response (trace b) did not evoke a Na⁺ spike. Figure 4C2 shows the result of digitally subtracting the average of the four smaller responses (trace a) from the average of the four larger responses (trace b) to reveal the shape of the potential that was evoked in an all-or-none manner. This potential resembles the iontophoretically evoked Ca²⁺ spike of Fig. 1D2 in shape, amplitude, and duration. Figure 4C3 shows the average of the four smaller responses (trace a) evoked in Fig.
FIG. 4. Ca\(^{2+}\) spike evoked by synaptic stimulation. Data from cell of Figs. 1 and 3. A: superimposed oscilloscope records showing membrane potential response (top) to intrasomatic hyperpolarizing current pulse (bottom) during which a pair of local, extracellular stimuli were applied at 29 \(\mu\)A. Arrows labeled 1 point to stimulus artifacts on 2 superimposed, sequential sweeps of cathodal stimulus pairs. Response to 2nd cathodal stimulus was much larger on 1 sweep (trace b). Arrows labeled 2 point to shock artifacts of anodal stimulus pairs of same strength that evoked no response. B: superimposed oscilloscope records showing 10 consecutive responses to cathodal stimulus pairs evoked during same hyperpolarization as A but shown at faster sweep speed. Second EPSP evoked a larger subthreshold potential (trace b) in all-or-none manner. During 2 sweeps, a low threshold Na\(^+\) spike (trace c, spikes clipped) arose from this larger potential. C1: superimposed digitized records of 8 responses to same cathodal stimulus pairs; 2nd EPSP evoked the larger potential (trace b) during 4 of these sweeps. C2: digital subtraction of average of 4 smaller responses (trace a) from average of 4 larger responses (trace b) in C1 reveals shape of all-or-none potential evoked by 2nd EPSP (cf. record of Fig. 1D2). C3: average of 4 smaller responses in C1 (trace a) to which average of 4 anodal stimuli were added to partially eliminate stimulus artifacts and better reveal the rising phase of EPSPs. Vertical calibrations in B (20 mV, 4 nA) apply to A and B; horizontal, 40 ms (A) and 10 ms (B); calibrations in C, 10 mV, 10 ms.

4C1. They have the shapes expected for EPSPs and they clearly differ from the all-or-none response of Fig. 4C2. From these results we conclude that the EPSPs evoked a Ca\(^{2+}\) spike that corresponds to the Ca\(^{2+}\) spike evoked by iontophoresis at the same site.

It is conceivable that the all-or-none fluctuation of the second evoked response in Fig. 4 results from a subset of presynaptic elements that are excited intermittently. The use of two extracellular stimuli provided a control for this possibility. We would expect to see an equally frequent fluctuation of the first of the two responses if intermittent stimulation of presynaptic elements were the cause of the fluctuations, but a similar fluctuation of the first response was never observed. Only the second response displayed the large, all-or-none fluctuation. On the other hand, the fluctuation might occur if the EPSP evoked by the second stimulus intermittently experienced paired-pulse facilitation. Arguing against this possibility is the fact that we were able to evoke the same all-or-none fluctuations by using a single stimulus of higher strength (data not shown).

In 5 of the 12 recorded cells we observed an all-or-none fluctuation of an EPSP-evoked potential that was similar to the Ca\(^{2+}\) spike evoked by iontophoresis in the same cell. Figure 5 shows an example from another cell in which iontophoresis evoked only a Ca\(^{2+}\) spike (Fig. 5A, / ). Figure 5B1 shows superimposed sweeps of the Ca\(^{2+}\) spike and the smooth, sub-threshold responses observed when the spike failed. The Ca\(^{2+}\) spike waveform is revealed in Fig. 5B2 by the digital subtraction of these sets of traces. In this cell it was not necessary to hyperpolarize the soma to observe all-or-none fluctuations of the electrically evoked response. Figure 5C1 shows 12 consecutive sweeps of the response to a pair of local extracellular electrical stimuli, the second of which evoked either small (trace 1) or large (trace 2) responses at constant stimulus strength. Figure 5C2 shows superimposed traces of the average of four of the larger responses and four of the smaller ones. As with the cell of Fig. 4, the all-or-none fluctuation of the response was seen only for the second EPSP. The result of digital subtraction of the smaller from the larger responses, shown in Fig. 5C3, is similar to the iontophoretically evoked Ca\(^{2+}\) spike in Fig. 5B2.

We observed previously that a Na\(^+\) spike evoked by depolarization of the soma occluded an iontophoretically evoked Ca\(^{2+}\) spike if the interval between the two spikes was short enough (Schwindt and Crill 1997). We performed this test on the EPSP-evoked Ca\(^{2+}\) spike in the cell of Fig. 5. In Fig.
In four sweeps the somatic depolarization was just subthreshold for somatic spike initiation. In 4 sweeps the somatic depolarization was subthreshold for somatic spike initiation (trace 1, left) and 2nd local stimulus evoked a large response (trace 1, right). On 1 sweep a somatic Na\(^+\) spike was evoked (trace 2, left, spike clipped during photography), and 2nd local stimulus evoked a smaller responses (trace 2, right). Note amplitude of 1st EPSP of the pair was not affected by somatic spike initiation. Vertical calibration 10 mV for A–D, 200 nA for A, 4 nA for D; horizontal, 40 ms (A), 10 ms (B and C), and 20 ms (D).

In three cells we investigated the possibility that current spread from the extracellular stimulation, rather than the postsynaptic EPSP, may have directly depolarized the postsynaptic membrane of the recorded cell to evoke the dendritic spikes. If this were the case, the extracellular stimulus would continue to evoke a postsynaptic depolarization and dendritic spikes even after postsynaptic glutamate receptors were blocked. We tested this idea by examining evoked responses before and after the application of APV + CNQX (50 \(\mu\)M each). Figure 6 shows the results from one of these cells. In this cell adequate iontophoresis evoked a sudden depolarization in an all-or-none manner that triggered two Na\(^+\) spikes (Fig. 6, A and B), the first of which was clearly a low-threshold spike (Fig. 6B; cf. current-evoked spike threshold in Fig. 6A). Repeating the iontophoresis during an adequate hyperpolarization of the soma revealed a Ca\(^{2+}\) spike (Fig. 6C, /) that could be triggered in an all-or-none manner. Apparently, the Ca\(^{2+}\) spike was responsible for the sudden membrane potential depolarization observed in the absence of somatic hyperpolarization. Addition of APV and CNQX to the perfusate rapidly eliminated the iontophotically evoked depolarization (Fig. 6D, trace 3). No depolarization could be evoked by iontophoretic currents up to \(-100\) nA (the largest that we could apply). This blockade of the iontophoretic response was reversible on washout of the glutamate receptor antagonists (Fig. 6E).
FIG. 6. Abolition of postsynaptic potentials by blockade of glutamate receptors. All records from same cell with piggyback electrode 423 μm from soma, Na⁺ spikes are clipped in all panels. A: superimposed records show spikes evoked all-or-none on consecutive sweeps by somatic current injection (left) and glutamate iontophoresis (right; −30 nA iontophoretic current). B: all-or-none iontophoretic response of A shown at faster sweep speed. Response consisted of sudden depolarization that evoked 2 Na⁺ spikes, the 1st of which was a low threshold (cf. current-evoked spike in A). Bottom trace: −76 mV; resting potential. C: iontophoresis during hyperpolarization of soma by injected current revealed Ca²⁺ spike (✓) that was evoked all-or-none. D: traces 1 and 2 are spike response and subthreshold depolarization, respectively, evoked by iontophoresis just before addition of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and N-2-amino-5-phosphonopentoic acid (APV; 50 μM each) to the perfusate. Trace 3 shows absence of response to same iontophoresis 3 min later. E: all-or-none iontophoretic response (evoked by −70 nA iontophoretic current) 6.5 min after return to physiological saline. F: EPSP amplitude increased until low-threshold Na⁺ spike was triggered as local extracellular stimulation was increased from 5 to 30 μA. G: local extracellular stimulation at 30 μA during soma hyperpolarization evoked smaller (trace 1) and larger (trace 2) response in all-or-none manner. Sixteen superimposed, consecutive traces are shown. ~1/2 small and 1/2 large. H: superimposed responses evoked at resting potential by local extracellular stimulation of 30 μA. Traces 1 (small responses), 2 (large responses), and 3 (low-threshold Na⁺ spike) are consecutive records taken just before addition of CNQX and APV (50 μM each) to the perfusate. Trace 4 shows absence of response to same stimulus 3 min later. I: 10 superimposed, consecutive records of responses evoked by local extracellular stimulus pair (70 μA) 6.5 min after return to physiological saline. Vertical calibration in A, 20 mV; 4 nA for injected current, 200 nA for iontophoretic current; applies to oscilloscope records of A, C, D, and E; horizontal in A, 200 ms for A and C and 100 ms for D and E; vertical in B, 20 mV for digitized records of B and F–I; horizontal in B, 40 ms (D) and 20 ms for B, F, H, and I.

The responses evoked by local extracellular stimulation were examined in between the iontophoretic trials. Figure 6F shows that the amplitude of the EPSP evoked by local extracellular stimulation was graded with stimulus amplitude, as was found in all cells tested in this study. The largest stimulus strength employed evoked a low-threshold Na⁺ spike similar to that evoked by iontophoresis (cf. Fig. 6, F and B). In addition, an all-or-none fluctuation of the amplitude of the synaptic response was apparent at a critical stimulus intensity, similar to the cells of Figs. 4 and 5. The fluctuation was clearest when the stimulus was applied during a hyperpolarization (Fig. 6G) that prevented the larger response (trace 2) from triggering Na⁺ spikes, but the fluctuations also were apparent at resting potential (Fig. 6H, traces 1–3). These potentials were completely abolished by the addition of APV and CNQX to the perfusate (Fig. 6H, trace 4). No response was observed even when a stimulus of 100 μA was employed (the largest tested). As with the iontophoretic response, the blockade was reversible on washout of the antagonists (Fig. 6I).

Similar results were obtained in the other two cells tested. One of these exhibited responses similar to the cell of Fig. 5, and the other exhibited only a low-threshold Na⁺ spike to both iontophoretic and local extracellular stimulation. We also performed control experiments to ensure that the glutamate receptor antagonists were not also blocking spikes be-
cause of nonspecific, “local anesthetic” effects at the concentrations used. Both Na⁺ spikes and their afterhyperpolarizations, evoked by depolarization of the soma, were unchanged in the presence of the antagonists, as were Ca²⁺ spikes evoked by somatic depolarization in the presence of TTX and 10 mM TEA chloride (data not shown). From these results we conclude that the dendritic spikes were evoked by the dendritic depolarization provided by the glutamatergic EPSPs, not by direct depolarization of the dendrite arising from spread of current from the extracellular stimulating electrode.

The cell of Fig. 6 was one of three cells in which the effects of local extracellular stimulation and bipolar stimulation of the pial surface (see METHODS) were compared. It was expected that the pia stimulation would excite (at least) afferent fibers in layer 1 that would in turn excite the distal dendrites of the recorded cell (Cauller and Connors 1994). Interestingly, the pia stimulation did not trigger a low-threshold Na⁺ spike in this cell, nor was any fluctuation apparent in the amplitude of the pia EPSP (which also was blocked by the glutamate receptor antagonists; data not shown). A second cell tested with both types of stimuli exhibited a low-threshold Na⁺ spike only in response to the local stimulus. The third cell responded differently and is described in the following section. These results are consistent with the idea that dendritic spikes are more readily evoked at specific dendritic sites.

Dendritic spikes evoked by summation of subthreshold potentials

In three cells we were unable to evoke dendritic spikes (neither low-threshold Na⁺ nor Ca²⁺ spikes) by EPSPs alone, perhaps because the stimulated afferent fibers impinged on a different part of the dendrite or were otherwise unable to adequately depolarize the region that gave rise to the iontophoretically evoked spike. In these cells we summed an otherwise ineffective EPSP with a subthreshold iontophoresis. We reasoned that the subthreshold dendritic depolarization caused by the iontophoresis might sum with the EPSP and thereby allow the effective dendritic region to reach spike threshold. Although this dendritic spike would not be caused solely by the EPSP, a positive result would show that synaptic input could evoke a dendritic spike when the dendrite was adequately depolarized by other means. Figure 7 shows an example. When applied at resting potential, the iontophoresis in this cell resulted in a sudden depolarization of membrane potential that evoked a Na⁺ spike (Fig. 7A, trace 1). This was not a low-threshold Na⁺ spike; its threshold was similar to that evoked by intrasomatic current injection (data not shown) and to the spike evoked by glutamate iontophoresis near the soma of the same cell (Fig. 7B).

This was one of two cells in which we compared dendritic and somatic glutamate iontophoresis. In practice, we placed the piggyback electrode within 35 μm of the soma and lowered it to the depth of the intracellular recording electrode. In both cells tested, the somatic iontophoresis evoked only a smoothly graded depolarization with no sign of the spike responses observed in the dendrites of the same cells. When the iontophoretic strength was sufficient, a spike was evoked whose threshold was similar to that evoked by injected current. Adequate local extracellular stimulation at the same point evoked an EPSP large enough to trigger a spike whose threshold was similar to that evoked by the iontophoresis (data not shown).

When the soma was hyperpolarized in Fig. 7A (trace 2) and the dendritic iontophoresis was repeated, the initiation of a Ca²⁺ spike became clear. (A capacitive coupling artifact from the break of the iontophoretic current occurs during this spike.) Apparently the Ca²⁺ spike was responsible for the sudden membrane potential depolarization observed in the absence of somatic hyperpolarization (Fig. 7A, trace 1). In this cell adequate local electrical stimulation evoked only a Na⁺ spike whose threshold was the same as for somatic current injection, and fluctuations of the underlying EPSP could not be detected even when it was evoked during somatic hyperpolarization (data not shown). However, when local extracellular stimulation was applied early during the iontophoresis, before the iontophoretically evoked Ca²⁺ spike occurred, the EPSP evoked by the extracellular stimulus triggered a Ca²⁺ spike in an all-or-none manner as shown in Fig. 7C. The stimulus artifact is marked by the asterisk in Fig. 7C. On alternate sweeps the stimulus evoked a Ca²⁺ spike (trace 1 on left) or a subthreshold EPSP (trace 2 on left). The EPSP-evoked Ca²⁺ spike occluded the iontophoretically evoked Ca²⁺ spike (trace 1 on right). When the extracellular stimulus evoked only a subthreshold EPSP (trace 2 on left), the iontophoretically evoked spike was unaffected (trace 2 on right). In addition, the iontophoretically evoked Ca²⁺ spike occluded the EPSP-evoked Ca²⁺ spike. In Fig. 7D the strength of the local extracellular stimulus was increased to evoke a Ca²⁺ spike on every sweep (not shown). When this stimulus (Fig. 7D, asterisks) was delayed until it occurred after the iontophoretically evoked Ca²⁺ spike (marked by arrow), the stimulus evoked only an EPSP. Notice that the iontophoresis evoked only the Ca²⁺ spike during these sweeps, not the Na⁺ spike. Thus the occlusion of the electrically evoked Ca²⁺ spike was caused solely by the previous iontophoretically evoked Ca²⁺ spike.

These results indicate unambiguously that the synaptically evoked Ca²⁺ spike is a postsynaptic event generated in the same dendritic area as the iontophoretically evoked Ca²⁺ spike. We verified in all recorded cells, including those in which EPSPs evoked dendritic spikes directly, that the summation of a subthreshold EPSP and a subthreshold iontophoresis could evoke a spike similar to that evoked by adequate iontophoresis alone or by the EPSP alone. Occlusion tests were performed in four of these cells, and all four tests gave results like those of Fig. 7, C and D.

We reasoned that if a subthreshold iontophoresis and a subthreshold EPSP could sum to evoke a dendritic spike, two independent subthreshold EPSPs occurring on the apical dendrite also should sum to evoke a dendritic spike. This idea was tested by employing bipolar electrical stimulation at the pial surface (see METHODS) in addition to the local extracellular electrical stimulation through the piggyback electrode. This test was performed on the cell of Figs. 8 and 9. Figure 8 shows some properties of the iontophoretically evoked spikes in this cell. Dendritic iontophoresis evoked a low-threshold Na⁺ spike that arose abruptly from a small, smooth, underlying depolarization (Fig. 8A, trace 1). Figure
**FIG. 7.** Ca\(^{2+}\) spike evoked by summation of subthreshold EPSP and iontophoresis and its interaction with iontophoretically evoked Ca\(^{2+}\) spike. A: 2 superimposed sweeps showing membrane potential responses to iontophoresis 444 μm from soma. Iontophoresis evoked an abrupt depolarization that triggered a Na\(^{+}\) spike (trace 1) when applied at resting potential (−80 mV). When applied during somatic hyperpolarization (trace 2), a Ca\(^{2+}\) spike was evoked. Capacitive artifact from break of iontophoretic current is superimposed on Ca\(^{2+}\) spike. Time derivative of Na\(^{+}\) spike (dV/dt) is shown for comparison with dV/dt in B. B: Na\(^{+}\) spike evoked all-or-none by iontophoresis near soma of same cell. C: 2 superimposed, consecutive sweeps showing evoked membrane potential responses when iontophoresis was applied during hyperpolarization of soma. Local synaptic stimulus of 9 μA applied early during dendritic glutamate iontophoresis (asterisk marks stimulus artifact) evoked either a Ca\(^{2+}\) spike (trace 1, left) that occluded the late iontophoretically evoked Ca\(^{2+}\) spike (trace 1, right) or a subthreshold EPSP that did not (trace 2). D: when applied after the iontophoretically evoked Ca\(^{2+}\) spike, larger local extracellular stimuli of 11 μA (asterisks) failed to evoke Ca\(^{2+}\) spikes. All Na\(^{+}\) spikes clipped during photography. Vertical calibrations, 20 mV and 200 nA for iontophoretic current, 4 nA for injected current, 1,000 V/s apply to A–D; horizontal, 100 ms applies to A–D.

8A, trace 2, shows the response to an intrasomatic injected current pulse. The difference in Na\(^{+}\) spike thresholds are clearer in the like-numbered traces of Fig. 8B, where traces of the all-or-none response evoked by the somatic current pulse (trace 2) and the iontophoretically evoked Na\(^{+}\) spike (trace 1) are superimposed. Typical of most cells that displayed a low-threshold Na\(^{+}\) spike, a large somatic hyperpolarization was required to block the iontophoretically evoked Na\(^{+}\) spike (Fig. 8, C and D, trace 1) and reveal an underlying Ca\(^{2+}\) spike (Fig. 8, C and D, trace 2).

Figure 9 shows the responses evoked by EPSPs in this cell. When adequate local or pia stimuli were applied at resting potential a low-threshold Na\(^{+}\) spike was evoked in this cell (data not shown). Other interesting properties of the EPSP-triggered responses were revealed when EPSPs were evoked during soma hyperpolarization. Figure 9A, trace 1, shows the stimulation paradigm employed. Paired local extracellular stimuli were applied during somatic hyperpolarization to evoke a pair of EPSPs. Also superimposed in Fig. 9A (trace 2) is the all-or-none response to a somatic injected current pulse. This trace is shown to contrast the threshold of the somatically evoked Na\(^{+}\) spike with the threshold of the dendritic Na\(^{+}\) spike. The traces in Fig. 9B show at faster sweep speed the EPSPs evoked during the same hyperpolarization as in Fig. 9A. Two consecutive responses to the same stimulus pair are shown superimposed. The subthreshold response (trace 1) is the same one shown in Fig. 9A. During the next sweep the same stimulus evoked a low-threshold Na\(^{+}\) spike (Fig. 9B, trace 2). The apparent threshold of this spike may be compared with that evoked by the current pulse in Fig. 9A, trace 2. Figure 9, C and D, is arranged similarly but shows the responses to stimulus pairs applied to the pia surface.

Figure 9, E and F, shows the second EPSP evoked by a pair of pia stimuli at faster sweep speed. These stimuli were applied at a higher strength and during a larger somatic hyperpolarization than in Fig. 9, C and D. Under these conditions, small all-or-none responses (Fig. 9, E and F, arrows) were evoked by the second EPSP (only) on about every second or third sweep. The fast rising and decaying response in Fig. 9E was seen most frequently and the slower rising and decaying
response of Fig. 9J less frequently. This latter waveform has the shape of the Ca$$^{2+}$$ spike of Fig. 8D, trace 2, but a smaller amplitude. It is apparent that the low-threshold Na$$^+$$ spike is triggered all-or-none by these smaller all-or-none events. Similar responses were not seen during local extracellular stimulation at any stimulus strength used up to spike threshold (data not shown). Only a subthreshold EPSP or a low-threshold Na$$^+$$ spike could be evoked by the local stimulation. These observations suggest that the pia stimulation triggered dendritic spikes at a more remote site than the local stimulation, and these remote dendritic spikes occasionally triggered a dendritic Na$$^+$$ spike that propagated to the soma.

Although local extracellular stimulation could not evoke the small, all-or-none potentials evoked by pia stimulation, the summation of a subthreshold local EPSP and subthreshold pia EPSPs could trigger both these smaller all-or-none potentials and the low-threshold Na$$^+$$ spike itself. When evoked alone, the pia EPSPs (Fig. 9G) and the local EPSP (Fig. 9, H and I) were subthreshold for any active response. The traces in Fig. 9J show the results of summing these EPSPs. These records were recorded consecutively at the same membrane potential, but trace 2 was shifted upward during photography for clarity. Figure 9J (trace 1) shows a small, all-or-none potential (arrow) that was triggered by the summed EPSPs during one of the sweeps. This response was similar to the one evoked by pia stimulation alone in Fig. 9E. On another sweep the summed EPSPs triggered the low-threshold Na$$^+$$ spike (Fig. 9J, trace 2). These types of responses alternated randomly from sweep to sweep at the stimulation strengths employed.

By comparing the amplitude of the summed subthreshold EPSP in Fig. 9J, trace 1, with the amplitudes of the EPSPs evoked separately in Fig. 9, G–I, it can be verified from the figure that the EPSP summation in Fig. 9J is almost perfectly algebraic. This is significant because the rising phases of the local EPSP and the second pia EPSP coincide in Fig. 9J. This timing would result in maximal shunting (and therefore imperfect summation) because of the associated synaptic conductance increase if the two sets of EPSPs were generated at the same dendritic locus. It is thus likely that each set of EPSPs arose in different dendritic regions of the apical dendrite, as expected because of the different sites of stimulation. Nevertheless, the depolarization provided by the spatial summation of the two sets of EPSPs was adequate to evoke dendritic spikes at sites that were inaccessible to the local EPSP alone. These results support the idea that subthreshold dendritic depolarizations, whether evoked by iontophoresis or EPSPs, can sum to evoke a dendritic spike.

**DISCUSSION**

The main conclusion that we draw from our observations is that the initiation of dendritic spikes is not unique to the use of glutamate iontophoresis. The stimulation of subsynaptic receptors, which is the cause of the EPSPs, is capable of evoking the same type of spikes. The ability of the EPSPs to evoke low-threshold Na$$^+$$ spikes provided the clearest evidence because the apparent threshold of these spikes was so far below that of spikes evoked by somatic depolarization and therefore so distinct. By performing occlusion tests we
showed that the synaptically and iontophoretically evoked Na⁺ spikes arose in the same area of the dendrite. Because they can resemble EPSP waveforms, the synaptic initiation of the Ca²⁺ spikes was harder to demonstrate. In identifying synaptically evoked Ca²⁺ spikes, we relied predominantly on the fact that small, all-or-none potentials triggered by the EPSPs had the same shape and amplitude as the Ca²⁺ spikes evoked by iontophoresis at the same dendritic site. But we also showed that these synaptically evoked Ca²⁺ spikes could be occluded by somatic Na⁺ spikes, and they could occlude and be occluded by the iontophoretically evoked Ca²⁺ spikes.

The ability of an EPSP to evoke dendritic spikes in layer 5 pyramidal neurons was shown by Kim and Connors (1993) in experiments employing tight-seal recording from the apical dendrite under experimental conditions similar to ours. They found that EPSPs evoked by electrical stimulation of layer 1 could trigger fast and slow active responses during dendritic recording. The shapes of these EPSP-triggered active responses were similar to those evoked by intradendritic depolarizing current pulses and identified pharmacologically as Na⁺ spikes and Ca²⁺ spikes. Because Kim and Connors (1993) recorded only from the dendrite, it was not clear if the EPSP-triggered Ca²⁺ spikes could be seen at the soma or if the EPSP-triggered Na⁺ spikes were backpropagated or arose in the dendrite. Our present experiments are complimentary to theirs in showing that EPSP-triggered, low-threshold Na⁺ spikes do arise in the dendrite.

We could not evoke dendritic spikes directly by local extracellular electrical stimulation in every cell tested. This is hardly surprising if the EPSPs need to depolarize the
same dendritic area as the iontophoresis. The local electrical stimulation raised the chances that the EPSPs would impinge on the same dendritic area, but by no means guaranteed this outcome. We always were able to evoke the dendritic spikes by pairing a subthreshold EPSP with a subthreshold iontophoresis, and we ascribed the success of this procedure to simple summation of the depolarization provided by each input at the effective dendritic site. This interpretation was supported by our finding that summation of two independent EPSPs could do the same thing. The latter experiment also demonstrated that EPSPs arising from conventional, gross electrical stimulation can also trigger dendritic spikes. In one of the cells tested, adequate stimulation at the pia not only evoked a low-threshold Na+ spike but also all-or-none potentials that appeared to represent spikes arising in more distal dendritic regions. Electrically evoked EPSPs are artificial compared with the natural synaptic activation that occurs during normal neural function, but our results at least raise the possibility that natural synaptic activation also can trigger dendritic spikes.

Evidence was presented in our previous study (Schwindt and Crill 1997) that the small, slow spikes and the large, fast spikes are initiated in the dendrites and are generated by Ca2+ influx and Na+ influx, respectively, through voltage-gated channels. We have assumed, without detailed examination, that the similar spikes evoked in this study also arose in the dendrites and had the same ionic nature. This seems a safe assumption given the unique electrical properties of these spikes. We also have assumed that the synthetically evoked depolarizations were pure EPSPs, although it is possible that they consisted at least partly of reversed IPSPs because our recording electrodes contained KCl. Practically speaking, this would make no difference to our conclusions because the depolarizations would still arise from the stimulation of subsynaptic receptors. However, we found that the application of 10 μM bicuculline affected neither the response evoked by dendritic iontophoresis nor the shape of the EPSP evoked by local electrical stimulation at the same site (n = 2) (unpublished observations), whereas glutamate receptor antagonists abolished the EPSPs (Fig. 6).

A series of recent experiments on different types of neurons have provided strong evidence that in many types of neurons Na+ spikes normally are initiated downstream from the soma but backpropagate into the dendrites (reviewed in Stuart et al. 1997). Other studies, mostly on hippocampal pyramidal cells, have presented evidence that synaptically evoked Na+ spikes can arise in the dendrites under some circumstances (Colling and Wheal 1994; Poolos and Kocsis 1993; Regehr et al. 1993; Turner et al. 1991). In some of these studies it seemed possible that the dendritic Na+ spikes were triggered by orthodromic stimuli that may have first evoked a downstream spike if not prevented from doing so in some manner. Recently a preliminary report of a study that used direct, tight-seal recording from the apical dendrite of mature, warmed neocortical neurons stated that Na+ spikes do arise first in the dendrite during adequate orthodromic depolarization, but that the spike that is propagated down the axon actually arises downstream from the soma (Stuart and Sakmann 1996). The large depolarization caused by a dendritic Na+ spike, perhaps propagating toward the soma in the proximal dendrite, could trigger a spike at the usual, more-excitatory downstream site, just like a large dendritic EPSP would. In this case the question of the precise point where the axon spike originated seems purely academic. The effect is the same as if the spike arose in the dendrite and propagated through dendrite, soma, and axon.

Considering only the events seen near resting potential in cells like that of Fig. 1, one could imagine that the Ca2+ spike sometimes is large enough to reach threshold of the usual downstream spike initiation site and triggers a Na+ spike there. Because the rising phase of the Ca2+ spike also is fast, perhaps the low-threshold Na+ spike merely appears to arise out of the baseline depolarization instead of arising from the Ca2+ spike at the normal somatic firing level. This idea is refuted by the fact that the low-threshold Na+ spike can be triggered by dendritic depolarization when the soma is hyperpolarized by 20 mV (thus somatic membrane potential is 40-mV negative to the usual firing level). On the basis of these and other observations, we have concluded that the low-threshold Na+ spikes normally are initiated in the dendrite. However, it is perfectly conceivable that, as explained earlier, the depolarization provided by the propagating dendritic Na+ spike triggers a spike at the downstream site. This secondary spike initiation could happen so fast that a corresponding electrical sign (such as an inflection on the spike) is impossible to detect.

One idea that our data refute, however, is that only a strong, suprathreshold synaptic stimulus evokes the dendritic Na+ spike. In those cells where synaptic stimulation evoked a low-threshold Na+ spike, the smallest EPSP that evoked any spike also evoked the low-threshold Na+ spike. It is possible that this result was so clear in our (successful) experiments because the local EPSPs depolarized only the spikes generating dendritic region. In hippocampal pyramidal neurons it was observed that the apparent threshold of spikes evoked by distal EPSPs was lower than that of proximal EPSPs (Andersen et al. 1987), but the difference in apparent threshold was, on average, smaller than we observed. Conventional gross electrical stimulation may sometimes evoke larger EPSPs at less-excitable regions of the neuron that trigger a Na+ spike in the usual manner. This need not always be the case, however, as demonstrated by our results using pia stimulation.

In contrast to cells that displayed a low-threshold Na+ spike, it was much easier to synaptically evoke the Ca2+ spike in the cells that displayed only the Ca2+ spike during iontophoresis. This occurred most frequently at the more distal dendritic sites (≥400 μm from the soma), and the Ca2+ spike could be evoked by an EPSP that was subthreshold for Na+ spike initiation at resting potential. This result is expected if the EPSP directly depolarized the Ca2+ spike-generating site. Again, the dendritic spike, a Ca2+ spike in this case, was evoked by a small, subthreshold EPSP, not by an EPSP that was suprathreshold for the usual downstream site of Na+ spike initiation.

On the other hand, in most cells where an EPSP evoked a low-threshold Na+ spike (e.g., the cell of Figs. 1, 3, and 4), we needed to evoke a larger EPSP to observe the Ca2+ spike. This larger EPSP would have triggered a Na+ spike if it was evoked from resting potential. To explain this observation we may suppose that the stimulated presynaptic fibers synapsed on the region where the Na+ spike was generated,
but that the Ca\textsuperscript{2+} spike was generated at a more distal region. This supposition would be consistent with our finding that more distal iontophoresis evokes only the Ca\textsuperscript{2+} spike. This idea is also consistent with the observation that the Na\textsuperscript{+} spike was blocked first during somatic hyperpolarization. A more distally originating potential would better survive somatic hyperpolarization. According to this idea, we would need to increase EPSP amplitude in cells like the one in Figs. 1, 3, and 4 to adequately depolarize this more distal region to Ca\textsuperscript{2+} spike threshold.

A more proximal dendritic location for low-threshold Na\textsuperscript{+} spike initiation also is suggested by the fact that the spike could be inactivated by adequate depolarization of the soma (Fig. 2). The effective depolarization was still below the threshold of the spike evoked by somatic depolarization. Operationally, this means that a synchronically evoked, low-threshold Na\textsuperscript{+} spike could not be observed unless the cell had a sufficiently high resting potential. This requirement may account in some part for the scarce reports of low-threshold, synchronically evoked spikes in previous studies. Functionally, this observation suggests that the low-threshold Na\textsuperscript{+} spike represents a transient response to sudden synaptic depolarization from an adequate resting potential; this response evaporates during sustained depolarization.

Why then is a presumably more distally initiated Ca\textsuperscript{2+} spike often or always associated with the low-threshold Na\textsuperscript{+} spike evoked by iontophoresis? We have presented evidence previously that dendritic depolarization activates an inward, persistent Na\textsuperscript{+} current (Schwindt and Crill 1995, 1996). The resultant voltage-gated inward membrane rectification would tend to lengthen the effective dendritic space constant so that 1) a larger area of the dendrite is depolarized and 2) dendritic events are more easily seen at the soma. In addition, dendrites of hippocampal pyramidal cells possess an inactivating, A-type K\textsuperscript{+} current (Hoffman et al. 1997). If a similar K\textsuperscript{+} current resides in neocortical dendrites, it would inactivate during the long-lasting depolarization provided by iontophoresis, again lengthening the effective dendritic space constant. An EPSP may provide too brief a depolarization to adequately evoke the voltage-gated inward rectification or to cause significant K\textsuperscript{+} current inactivation, so that a larger proximal EPSP would be needed to evoke the Ca\textsuperscript{2+} spike at a more distal site. The relation between the Ca\textsuperscript{2+} spike and the initiation of the Na\textsuperscript{+} spike needs further investigation.

We have seen in the present study that it is difficult or impossible to recognize that an EPSP evoked a dendritic Ca\textsuperscript{2+} spike by simple inspection of the evoked potential. We proposed (Schwindt and Crill 1997) that the large Ca\textsuperscript{2+} influx shown to occur through voltage-gated Ca\textsuperscript{2+} channels during subthreshold EPSPs (Magee et al. 1995; Markram and Sakmann 1994) may occur because the EPSP triggered a local Ca\textsuperscript{2+} spike that was indistinguishable from a pure EPSP when viewed from the soma. Our present results give more credence to this idea, and it seems to be supported by a preliminary report of a study that employed direct dendritic recording and Ca\textsuperscript{2+} imaging in mature layer 5 pyramidal cells (Schiller et al. 1996). If this view is correct, one can imagine that widespread synaptic depolarization of the distal dendrites may evoke local Ca\textsuperscript{2+} spikes at multiple locations. The Ca\textsuperscript{2+} spike generated at each location would be small when viewed from the soma, and the potential generated by the summation of these multiple events may be indistinguishable from a pure EPSP. Because multiple, small Ca\textsuperscript{2+} spikes would contribute to this potential, one could not even expect to identify them by observing large, all-or-none fluctuations as we were able to do when evoking a Ca\textsuperscript{2+} spike at a single, relatively proximal site. Thus our present observations raise the possibility that any composite (multifiber) dendritic EPSP may be composed both of multiple small synaptic potentials and multiple small Ca\textsuperscript{2+} spikes. This probably would not apply to EPSPs evoked by one or a few afferent fibers, because the dendritic depolarization caused by the postsynaptic current would be too small to activate voltage-gated Ca\textsuperscript{2+} channels, at least in larger diameter dendrites.

In our previous study (Schwindt and Crill 1997) we also presented several lines of evidence that the Ca\textsuperscript{2+} spike evoked in physiological saline was a ‘‘local,’’ nonactively propagated spike (i.e., its active generation was restricted to a discrete region of the distal dendrite). This idea also appears to have been supported by a study that employed direct dendritic recording and Ca\textsuperscript{2+} imaging in mature layer 5 pyramidal cells (Schiller et al. 1996). One of our observations was that consistent with the generation of a local Ca\textsuperscript{2+} spike that was smaller amplitude Ca\textsuperscript{2+} spikes were generated by iontophoresis at more distal dendritic sites (Schwindt and Crill 1997). We proposed that these local Ca\textsuperscript{2+} spikes serve the function of transiently boosting a sufficiently large synaptic depolarization at the site so that the synaptic input would have a larger influence (or any influence at all in the case of very distal synaptic input) in depolarizing the soma and the usual downstream spike initiation site. Modeling studies concluded that some active boost must occur for very distal input to influence the soma (e.g., Caulker and Connors 1992). A transient boost of adequate synaptic depolarization by a Ca\textsuperscript{2+} spike would still preserve, to some extent, the relative weighting of synaptic input that would occur in a passive dendrite, because Ca\textsuperscript{2+} spikes generated more distally are smaller (when viewed at the soma) than those generated more proximally. This idea, along with several others that we proposed in our previous study (Schwindt and Crill 1997) regarding the role of dendritic spikes in altering the cell’s input-output properties, gains more credence from the fact that we have shown that EPSPs can initiate dendritic spikes similar to those evoked by the glutamate iontophoresis.

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