0270-6474/82/0211-1614$02.00/0

The Journal of Neuroscience
Vol. 2, No. 11, pp. 1614-1622
November 1982

ELECTROPHYSIOLOGY OF ISOLATED HIPPOCAMPAL PYRAMIDAL DENDRITES[1]

LARRY S. BENARDO,[2] LEONA M. MASUKAWA,[3] AND DAVID A. PRINCE[4]

Department of Neurology, Stanford University School of Medicine, Stanford, California 94305

Received March 11, 1982; Revised May 24, 1982; Accepted May 24, 1982

Abstract

In order to study the electrical properties of dendritic membranes independent of the effects of somatic potentials, intracellular recordings in guinea pig hippocampal slices were obtained from the dendrites of CA1 pyramidal neurons (HPCs) which had been isolated from their somata by cuts made through the proximal stratum radiatum. Spikes and subthreshold membrane responses to intracellular current pulses were compared in intact and isolated dendrites and in the residual portions of neurons whose apical dendrites had been severed ("isolated somata"). Isolated dendrites generated both fast, QX-314-sensitive, sodium-mediated spikes, and slow higher threshold spikes which were QX-314-resistant and presumably mediated by Ca^{2+}. Depolarization of "isolated somata" ordinarily evoked only fast (Na^+) spikes, but presumed Ca^{2+} spikes could be elicited after exposure to QX-314 (a local anesthetic). Anomalous inward rectification was depressed by QX-314 in somata but not in dendrites, suggesting that the ionic basis for subthreshold as well as regenerative conductances was different at different sites on the neuron.

The dendritic membrane in CA1 HPCs thus generates both Na^+- and Ca^{2+}-mediated spike potentials and a subthreshold response which probably is mediated primarily by Ca^{2+}. Attempts to describe the integrative functions of these neurons must take into account the variety of conductances which are activated nonuniformly in somata and dendrites by changes in membrane potential.

The electrical properties of dendrites in mammalian cerebral neurons have been a key issue in the study of the CNS (Purpura, 1967; Llinas, 1975). Although it was suggested initially that the dendritic membrane was chemically, but not electrically, excitable (for review, see Purpura, 1967), subsequent data from neurons in the mammalian neocortex (Purpura and Shofer, 1964; Purpura and McMurtry, 1965; Purpura et al., 1965), hippocampus (Spencer and Kandel, 1961; Purpura et al., 1966), and cerebellum (Llinas et al., 1968; Llinas and Nicholson, 1971) suggested that dendrites were capable of action potential generation. The presence of ionic channels which mediate regenerative events in dendrites has many important implications for neuronal information processing (Purpura, 1967; Llinas and Nicholson, 1971). In addition, there has been much speculation regarding the role of active dendritic membranes in disease states, such as epilepsy (Purpura, 1969; Westrum et al., 1964; Wong and Prince, 1979).

The electrophysiology of dendrites has been addressed directly in two classes of CNS neurons through the use of intradendritic recordings. In mammalian Purkinje cells studied *in vitro*, distal dendritic recordings, which were not confounded by interference from potentials originating in the soma, showed that membranes were capable of generating slow Ca^{2+} spikes (Llinas and Hess, 1976; Llinas and Sugimori, 1980b). Intradendritic recordings from hippocampal pyramidal neurons (Wong and Prince, 1979; Wong et al., 1979; Schwartzkroin and Prince, 1980) have suggested that these elements may generate both Ca^{2+}- and Na^+-mediated action potentials. Unfortunately, it is difficult to determine whether signals recorded in the dendrite are generated locally or more remotely in the soma because of the short electrotonic length of hippocampal pyramidal neurons (Turner and Schwartzkroin, 1980; Brown et al., 1981).

In addition to the ionic conductances responsible for spike generation, the membranes of pyramidal neurons

[1] This work was supported by National Institutes of Health Grants NS 06477 and NS 12151 from the National Institute of Neurological and Communicative Disorders and Stroke and the Morris Research Fund. We wish to thank Jay Kadis for technical assistance and Cheryl Joo for manuscript preparation.

[2] Present address: Columbia University School of Medicine, Box 15, Bard Hall, 50 Haven Avenue, New York, NY 10032.

[3] Present address: Department of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

[4] To whom correspondence should be addressed.

possess other voltage-dependent subthreshold conductances for Na^+, Ca^{2+}, and K^+ (Hotson et al., 1979; Wong and Prince, 1981; Johnston et al., 1980). One important issue with respect to all of these subthreshold conductances is whether they are distributed uniformly over soma-dendritic membranes or whether the electrogenic properties in pyramidal dendrites and somata are fundamentally different.

In order to examine the above issues further, we studied the electrical properties of hippocampal pyramidal cell dendrites directly by recording from isolated apical dendrites after microsurgically separating these structures from their somata. The isolated dendritic preparation provided an unique opportunity to examine the distribution of certain voltage-dependent conductances on dendritic membranes.

Materials and Methods

The methods for preparing and maintaining hippocampal slices have been described elsewhere in detail (Yamamoto, 1972; Schwartzkroin, 1975). Experiments were performed at 37°C. Recordings from intact and isolated elements were made in the midportion of the CA1 region (CA1b). Following a 15- to 60-min period of incubation in the recording chamber, a single cut was made in the stratum radiatum 25 to 150 μm from the pyramidal cell body layer through the entire thickness of the slice in the CA1 region. To accomplish this, razor blade fragments were attached to microelectrode capillary glass with wax and the capillary then was affixed to a micromanipulator. Since the cell body layer of hippocampal pyramidal neurons (stratum pyramidale) is readily seen in the transilluminated slice, accurate placement of microknives and subsequent sections in the stratum radiatum could be performed easily under visual control. The completeness of surgical "cuts" was verified by examining cresyl violet-stained sections which showed that the lesions went through the entire thickness of the slice. Standard perfusion medium was used with the exception that the $CaCl_2$ concentration was increased to 2.5 mM in an attempt to improve recording stability and to aid "sealing" of neuronal processes. Following these procedures, we could obtain stable intracellular recordings from isolated dendrites for 5 to 30 min during experiments lasting up to 6 hr.

Microelectrodes were filled with 4 M potassium acetate or 4 M potassium acetate plus 0.1 M QX-314 (a local anesthetic; Astra) and had resistances of 30 to 60 megohms. Current was applied through the recording microelectrodes utilizing an active bridge circuit. Bridge balance was monitored and adjusted continuously. Membrane potentials (V_m) were measured by comparing the intracellular potential with that obtained after withdrawal from the impaled element. The apparent membrane input resistance (R_N) was determined by measuring the responses to small hyperpolarizing pulses at resting V_m.[5] The numbers and types of elements from which successful recordings were obtained and their properties are summarized in Table I.

[5] The membranes of hippocampal pyramidal neurons possess several voltage-dependent conductances which may be activated or inactivated by test current pulses. The term "apparent membrane input resistance" is used to indicate this.

Results

Spike generation in dendrites

Intact dendrites. Recordings from intact dendrites were obtained in the stratum radiatum of the midportion of the CA1 region at a distance of 100 to 350 μm from the margin of the stratum pyramidale. As previously reported (Wong and Prince, 1979; Wong et al., 1979), such recordings were easily distinguishable from those obtained in pyramidal cell bodies (see below) or from cells having the characteristics of interneurons (Schwartzkroin and Mathers, 1978). As shown in Table I, R_N was 33.6 ± 12.7 megohms (n = 8) at a resting V_m of 59.6 ± 4.7 mV (n = 8). These values are similar to those obtained previously from intact dendrites (Wong et al., 1979). We were able to evoke a variety of spike-like events with depolarizing current pulses as shown in Figure 1. Low intensity depolarization elicited only repetitive fast spikes (Fig. 1*A1*). However, with increased current, the threshold for generation of slower spikes could be reached (Fig. 1*A2*). Spikes evoked at or above this threshold had variable amplitudes and tended to occur in clusters (Fig. 1, *A2* to *A4*). A further increase in current triggered an increasing number of slower spikes intermixed with faster action potentials (Fig. 1*A3*). Spikes were grouped arbitrarily into three types, fast, intermediate, and slow, according to their duration at half-amplitude (Table I). The fast action potentials rose and decayed rapidly and had a duration of 0.94 ± 0.3 msec (n = 8) (Fig. 1*A4*, *arrow a*). The intermediate duration action potentials (Fig. 1*A4*, *arrow d*) also had a rapid rising phase but decayed more slowly (2.4 ± 0.8 msec; n = 8). The slow spikes (Fig. 1*B4*, *arrow b*) were characterized by both slow rising and falling phases and had a duration of 5.2 ± 1.3 msec. The occurrence of spikes of various durations appeared sometimes to overlap so that a second or third spike with different amplitude and duration might be triggered on the repolarizing phase of the initial spike (Fig. 1, *A4*, *arrows b* and *c*, and *B4*, *arrow a*). Spontaneous activity was present in dendrites and consisted of single fast spikes of various amplitudes as well as intermediate duration spikes. Fast prepotentials (Spencer and Kandel, 1961; Schwartzkroin, 1977; Schwartzkroin and Prince, 1978) less than 5 mV in amplitude also were observed and, in some instances, appeared to be generator potentials for fast spikes.

Isolated dendrites. In several instances, there was an obvious physical separation at the site of the cut visible in the recording chamber under the dissecting microscope. Completeness of the isolation procedure was verified in cresyl violet-stained sections. In all cases, the stratum radiatum had been separated completely from the cell body layers in the stratum pyramidale. The tissue on the somatic side of the cut thus presumably contained the basal dendrites, somata, and the proximal stumps of the apical dendrites of pyramidal neurons. An example of such a slice is shown in Figure 2. In this case, the cut was extended through the strata pyramidale and oriens to the alveolar surface and the separated portion was

TABLE I

Characteristics of the elements from which successful recordings were obtained

	Fast Spike		Intermediate Spike		Slow Spike		Resting V_m	R_N	n
	Amplitude	Duration	Amplitude	Duration	Amplitude	Duration			
	mV	*msec*	*mV*	*msec*	*mV*	*msec*	*mV*	*megohms*	
Intact dendrite	66.6 ± 8.1	0.94 ± 0.3	59.6 ± 5.8	2.4 ± 0.8	54.9 ± 10.9	5.2 ± 1.3	59.6 ± 4.7	33.6 ± 12.7	8
Isolated dendrite	63.7 ± 10.0	1.21 ± 0.3	54.6 ± 5.0	2.85 ± 0.5	44.9 ± 6.7	7.4 ± 1.59	57.4 ± 4.9	29.75 ± 15.6	8
QX-314-injected isolated dendrite					56.8 ± 9.0	8.0 ± 2.9	55.3 ± 5.5	23.3 ± 2.9	4
Isolated soma	73.3 ± 6.1						62.0 ± 3.5	43.3 ± 20.8	4

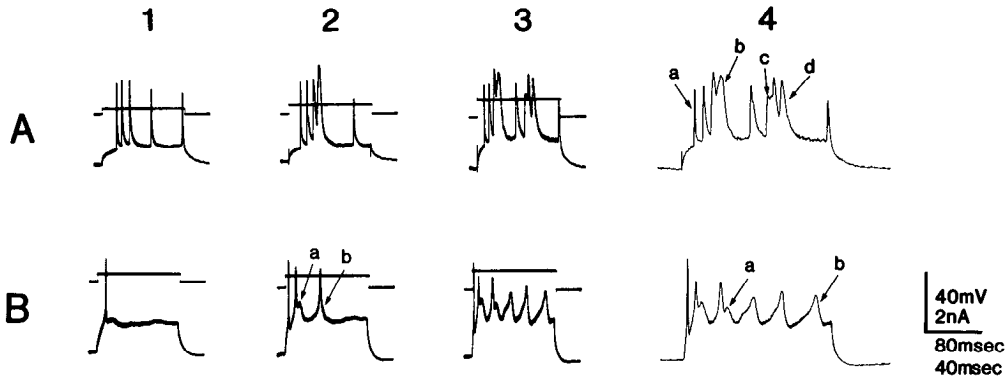

Figure 1. Spike generation in intact (*A*) and isolated (*B*) dendrites of CA1 pyramidal cells. Low intensity current pulses evoked fast spikes in intact (*A1*) and isolated (*B1*) dendrites. More intense stimuli evoked trains containing intermediate duration spikes in both intact (*A2* and *A3* and *arrow d* in *A4*) and isolated (*B2, arrow b*) dendrites. Further depolarization of isolated dendrite evoked a train of predominantly slow regenerative potentials (*B3* and *B4, arrow b*). Some spikes are composed of multiple components, with events occurring on the rising phase (*arrow c, A4*) and falling phase (*arrow b, A4* and *arrow a, B2* and *B4*) of the principal spike. The *traces* in *A4* and *B4* are the same as *A3* and *B3*, respectively, at a faster sweep speed. The *upper traces* in *frames 1, 2,* and *3* are a current monitor. The *calibration bars* in *B4* are for all of the frames except the 40-msec time calibration which is for *A4* and *B4.*

removed prior to recording. Most of the cell bodies seen in the stratum radiatum were those of glia. Recordings were obtained from that portion of the stratum radiatum where the overlying stratum pyramidale (marked by *triangles* in Fig. 2) was absent.

Approximately 15 to 60 min after the surgical procedures, dendrites could be impaled successfully; however, the number encountered in a given slice was greatly decreased after the cut had been made. Membrane potentials were initially low but gradually improved over the 1 to 2 hr after the isolation was performed. The data presented here were collected, after this early recovery period, from those dendrites which had initial membrane potentials greater than −50 mV and from which stable recordings could be maintained for from 5 to 30 min.

Isolated dendrites usually were impaled at least half the distance between the cut edge of the stratum radiatum and the hippocampal fissure. The mean values for resting V_m and R_N were comparable to those in intact dendrites (Table I). In contrast to recordings from intact dendrites, spontaneous spikes were absent in isolated dendrites as were fast prepotentials. The latter finding suggests that the fast prepotentials recorded in dendrites of intact neurons are reflections of the fast spikes generated in somata or more proximal dendritic loci. Spontaneous excitatory synaptic events which increased in amplitude with hyperpolarization were present in isolated dendrites. Action potentials could be elicited with depolarizing current pulses. The response to threshold current consisted of a distinct single fast spike that was comparable to the fast spike seen in intact dendrites (Fig. 1*B1*; Table I). Isolated dendrites generally did not generate trains of fast spikes during depolarization in contrast to intact elements (cf., Fig. 1, *A1* and *B1*). With higher current intensities, higher threshold intermediate duration spikes were observed (*arrow b* in Fig. 1*B2*). As in the case of intact dendrites, a second overlapping spike sometimes might arise from the falling phase of a preceding one (*arrow a* in Fig. 1, *B2* and *B4*). At still higher current intensities, trains of slow spikes were evoked and followed by slow afterhyperpolarizations (AHPs) (Fig. 1*B4*) which resembled the AHPs attributed to a Ca^{2+}-activated K^+ conductance in CA1 hippocampal pyramidal cell (HPC) somata (Hotson and Prince, 1980).

Support for the conclusion that dendritic membranes are capable of generating both fast (presumed Na^+) spikes and slow (presumed Ca^{2+}) spikes was obtained by blocking voltage-dependent Na^+ conductance with intracellular injections of the local anesthetic, QX-314. Recent experiments have shown that intracellular diffusion of this agent from the microelectrode into CA1 cells (Connors and Prince, 1982) or neocortical neurons (Connors et al., 1981) blocks Na^+-mediated spikes and the voltage-dependent non-inactivating slow Na^+ conductance present in these neurons. The effects of QX-314 are identical to those of externally applied tetrodotoxin (TTX), and

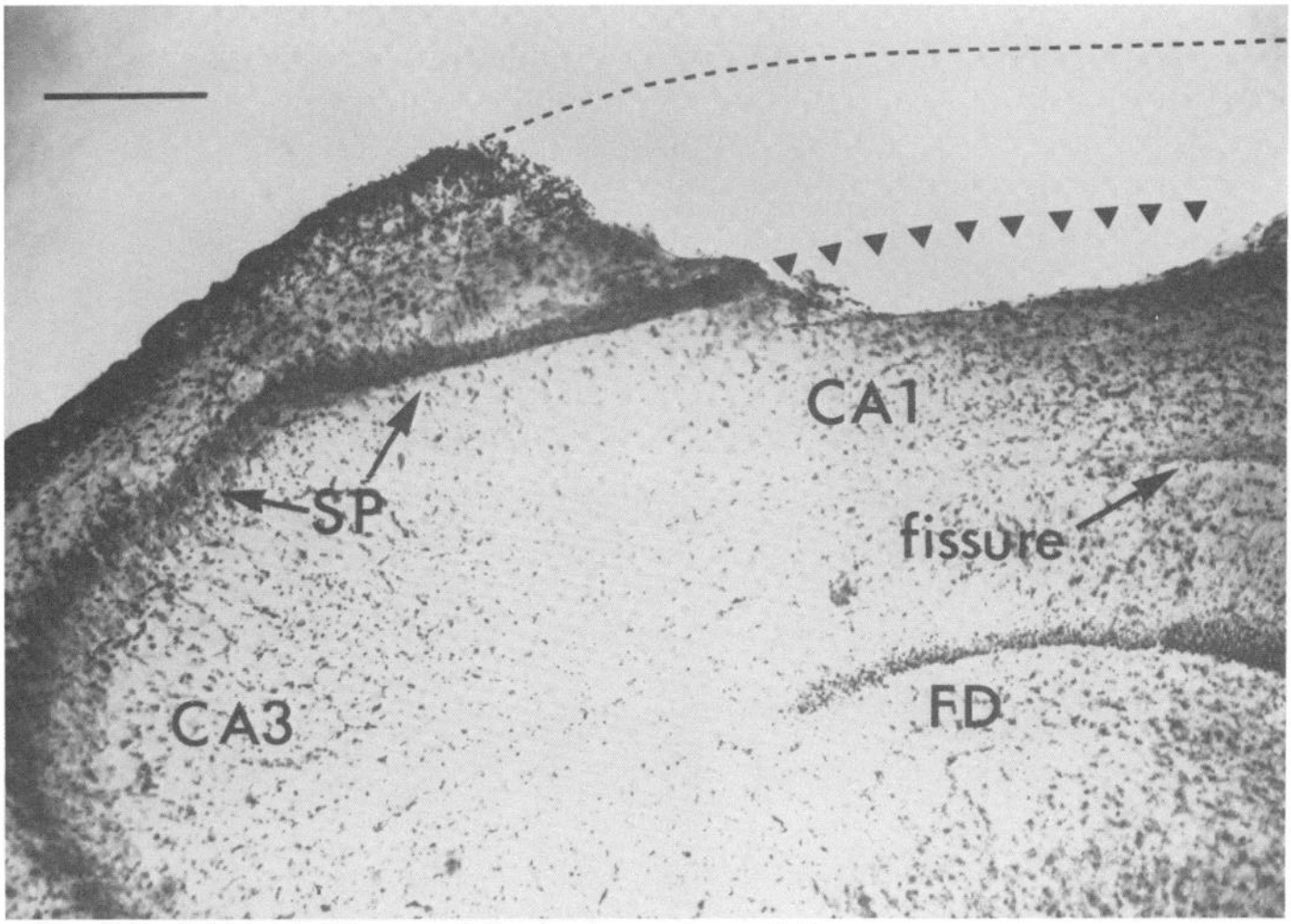

Figure 2. Cresyl violet-stained hippocampal slice after the cut and removal of a portion of the CA1 stratum pyramidale (*SP*) with the adjoining strata oriens and radiatum and alveus. The *solid triangles* and *dashed line* indicate the approximate respective positions of the pyramidal cell body layer and alveolar surface of the removed portion of the slice. The cut was made approximately 100 to 150 μm into stratum radiatum in the fresh state (prior to shrinkage due to fixation). Dendrites later were impaled between the cut and the hippocampal fissure. The abrupt angulation of the *SP* between the *arrows* is due to distortion from the cut extending into that portion of stratum pyramidale. *FD,* Dentate fascia. *Calibration bar,* 300 μm.

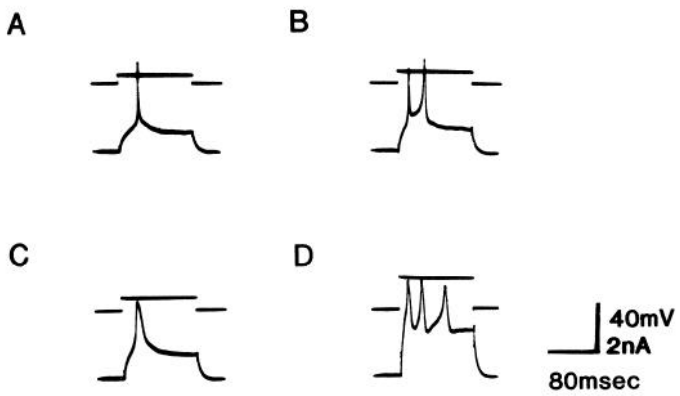

Figure 3. Effects of QX-314 injection on spikes in an isolated dendrite. Immediately after penetration with a QX-314-filled microelectrode, a fast spike was present (*A*) which was accompanied by an intermediate duration spike (*B*) when the depolarizing current was increased. Shortly after penetration, following the diffusion of QX-314 into the neuron, the fast spike was no longer present; however slow spikes could be elicited singly (*C*) or repetitively with increased current (*D*). The *calibration bars* in *D* are for all sweeps.

both of these agents produce effects on sodium-mediated events similar to those produced by media containing no Na^+.

Following impalement with microelectrodes containing 0.1 M QX-314 and 4 M potassium acetate, spikes were elicited with depolarizing current. Early in the impalement, single fast action potentials could be seen (Fig. 3*A*). These fast spikes often were followed by an intermediate duration spike (Fig. 3*B*). Within seconds, however, the fast spike disappeared, and a single slow spike was evoked by threshold current pulses (Fig. 3*C*). In other penetrations, blockade of fast spikes by QX-314 occurred only after passage of repetitive depolarizing current pulses. Inflections on the rising or falling phase of the slow spikes indicative of a superimposed spike were not seen in recordings from the four isolated dendrites exposed to QX-314. The parameters of slow spikes in QX-314-injected and noninjected dendrites were comparable (Table I). Cells injected with QX-314 also were noted to depolarize by about 5 mV. Once fast spikes were blocked, repetitive slow spikes could be evoked by increasing the current intensity (Fig. 3*D*). This result is

similar to that reported during the depolarization of intact somata (Schwartzkroin and Slawsky, 1977; Wong and Prince, 1977, 1978) and dendrites (Wong and Prince, 1979; Wong et al., 1979) of hippocampal pyramidal neurons following application of TTX. Intracellular injection of QX-314 into intact somata also blocks fast spikes and allows higher threshold slow spikes to be evoked (Connors and Prince, 1982; L. M. Masukawa, L. S. Benardo and D. A. Prince, unpublished observation). The data suggest that Na^+ action potentials can be generated by dendritic membranes and that QX-314 is effective in blocking these Na^+-dependent conductances in dendrites.

Spike generation in isolated somata

Isolated elements consisting of basal dendrites, cell bodies, and probably the most proximal portions of the apical dendritic tree, referred to below as "isolated cell bodies," also could be impaled. These elements were less viable than isolated dendrites; impalements could be obtained only within the first 3 hr following the surgical procedure. Isolated cell bodies often displayed abnormal membrane properties, such as large amplitude, prolonged afterhyperpolarizations, and unstable V_m and R_N, which decreased rapidly after impalement. However, some isolated cell bodies appeared to have normal spikes and membrane properties (Table I). When depolarizing pulses were delivered to isolated somata, only fast spikes could be elicited, regardless of the current intensity (Fig. 4, *A1* to *A3*). Trains of spikes were followed by slow afterhyperpolarizations lasting as long as 1 sec. The responses were similar to those of intact somata in the CA1b region to depolarizing current (Fig. 4, *B1* to *B3*).[6] Since high threshold Ca^{2+} spikes can be recorded in intact CA1 somata in the presence of blockers of Na^+ conductance, such as TTX (Schwartzkroin and Slawsky, 1977; Wong et al., 1979; Hotson and Prince, 1980), we studied the effects of QX-314 on isolated somata to determine whether Ca^{2+} spikes could still be evoked in the absence of the apical dendritic arborization. The results are shown in Figure 5. Shortly after impalement, the neuron generated full amplitude fast spikes (Fig. 5*A1*). Over minutes, the spikes decreased in amplitude and higher threshold slow spikes, which were presumably Ca^{2+}-mediated (see also Connors and Prince, 1982), could be elicited (Fig. 5, *A2* to *A3*). When a drop of Ba^{2+} (5 mM) was applied to the surface of the slice from a broken pipette near the penetrating microelectrode, input resistance increased (cf., sweeps of Fig. 5, *A1* and *B2*), and the slow spike increased in amplitude and broadened, presumably due to effects of Ba^{2+} as a Ca^{2+} agonist and blocker of K^+ conductance (Hagiwara et al., 1974; see also Hotson and Prince, 1981). These findings provided further evidence that the slow spikes generated after exposure to QX-314 were mediated by Ca^{2+}.

Subthreshold conductances

Subthreshold slow conductances for both Na^+ and Ca^{2+} have been identified in recordings from CA1 cell somata (Hotson et al., 1979). Inward curents caused by these ions are probably responsible for the apparent increase in resistance which accompanies the depolarization of these neurons (anomalous inward rectification (AR); Hotson et al., 1979). We studied the responses of isolated and intact dendrites and found that AR could be detected in these elements as well as in intact somata; that is, the responses to subthreshold depolarizing current pulses were larger in amplitude than those produced by comparable hyperpolarizing current pulses. However, there were variations in the time course of development of the depolarizing and hyperpolarizing responses as can be seen in Figure 6. The responses in intact dendrites (Fig. 6*B1*) and cell bodies (Fig. 6*A1*) were similar in that long depolarizing current pulses (~120 msec) elicited

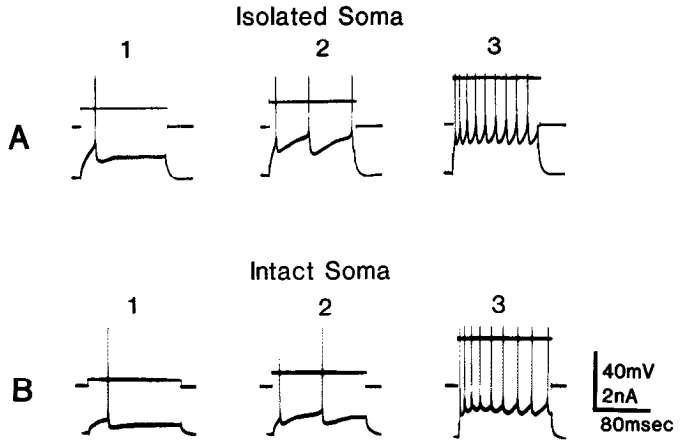

Figure 4. Spike generation in isolated (*A*) and intact (*B*) somata. Spike frequency increased with increasing depolarizing current in isolated soma (*A1* to *A3*); however, only fast spikes were observed even with high currents (*A3*). This same sequence also was seen in a majority of intact CA1 cell somata (*B1* to *B3*).

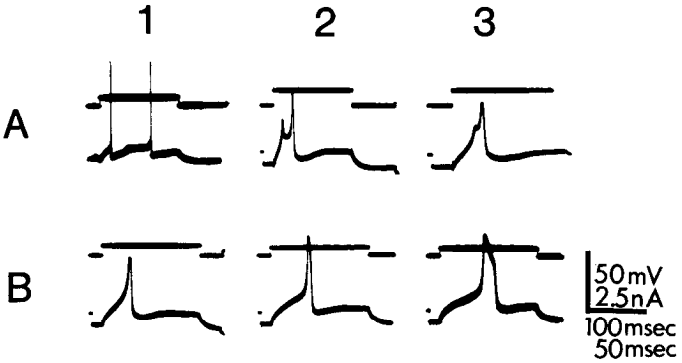

Figure 5. Effects of intracellular injection of QX-314 and extracellular application of Ba^{2+} to the "isolated soma" of a CA1 HPC. *A1*, Shortly after impalement, the neuron generates fast spikes during direct depolarization. *A2* and *A3*, Within 10 min, fast spikes are progressively blocked and higher threshold slow spikes are evoked. *B1*, Control spike from the same neuron 30 min after impalement. *B2*, Increase in spike amplitude occurred within 2 min of Ba^{2+} (5 mM) application. *B3*, Superimposed sweeps showing further spike broadening induced by Ba^{2+} 5 min after the sweep of *B2*. The *voltage* and *current calibration bars* in *B3* are for all sweeps. *Time calibration bar* in *B3*, 100 msec for *A1* and *A2* and *B*; 50 msec for *A3*.

[6] Burst responses containing slow spikes may be evoked by depolarization of the somata of pyramidal neurons in the CA1a and CA1c regions (L. M. Masukawa et al., manuscript in preparation).

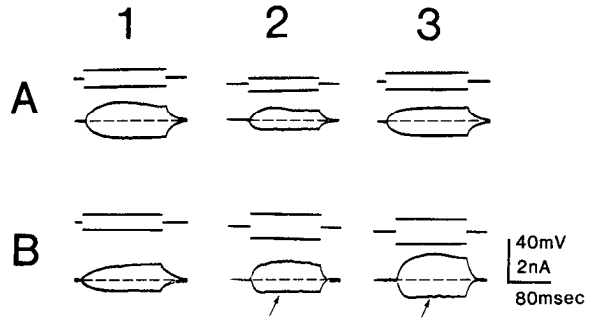

Figure 6. Rectification properties of somata and dendrites. Voltage responses to depolarizing and hyperpolarizing current pulses of equal intensity and opposite sign are shown in each sweep. Anomalous rectification to depolarizing current was observed in intact (*A1*) and isolated (*A2*) somata and intact (*B1*) and isolated (*B2*) dendrites. Hyperpolarizing currents produced potentials that plateaued (*A1, A2,* and *B1*); however, in isolated dendrites (*arrow, B2*), the current-evoked potential began to sag toward the resting potential after reaching an early maximum. After QX-314 injection, rectification to hyperpolarizing and depolarizing current was still present in isolated dendrites (*B3* and *arrow*), although fast spikes were absent (see Fig. 3). In intact somata, depolarizing anomalous rectification was lost after QX-314 (*A3*).

voltage responses which continued to rise either throughout the duration of a pulse (Fig. 6*B1*) or throughout most of its early portion before a latter "sag" developed (Fig. 6*A1*). This late sag may be due to either an inactivation of the voltage-dependent component of the depolarization or an activation of a delayed rectification. In contrast, hyperpolarizing pulses evoked responses which reached a steady state potential that was smaller in maximum amplitude than the response to depolarizing current (Fig. 6, *A1* and *B1*).

Isolated (Fig. 6*A2*) and intact (Fig. 6*A1*) cell bodies gave qualitatively similar voltage responses when tested for AR. The depolarizing pulse elicited a response which reached an early maximum and then gradually declined; in the same elements, the hyperpolarizing response reached a plateau that was smaller in amplitude than the depolarizing response (Fig. 6, *A1* and *A2*). In isolated dendrites, responses to depolarizing pulses were clearly of larger amplitude than those to hyperpolarizing pulses (Fig. 6*B2*). Hyperpolarizing current pulses evoked a maximum response early, followed by a subsequent reduction in the amplitude of the response seen as a sag in the voltage trace (*arrow* in Fig. 6*B2*). We have not analyzed this latter response further; however, it may be similar to the anomalous rectification described in cat spinal motoneurons (Nelson and Frank, 1967).

Although it has been reported on the basis of intrasomatic recordings that Mn^{2+} or TTX decrease AR in hippocampal CA1 pyramidal cells, the relative contributions of Ca^{2+} and Na^+ conductance to AR are not known. Recent observations in CA1 pyramidal neurons (Benardo and Prince, 1982) and in neocortical neurons (Connors et al., 1981) suggest that the AR noted in intrasomatic recordings is more sensitive to TTX than to Mn^{2+} application. The presumed slow inward current underlying AR in these cell bodies therefore may be carried primarily by Na^{2+}. Since the above data from isolated dendrites and somata suggested that Ca^{2+} spike generation was much more prominent in dendrites, we conducted experiments to determine whether this was also the case for the subthreshold Ca^{2+} conductance which contributes to AR.

Impalements of isolated dendrites were made with electrodes containing QX-314 (Table I). As shown in Figure 6*B3*, AR could still be observed in those dendrites even after fast spikes were blocked by diffusion of the drug. Further, the time course of voltage responses in such recordings was very similar to that seen in isolated dendrites impaled with 4 M potassium acetate-filled microelectrodes (cf., Fig. 6, *B2* and *B3*). By contrast, AR in intact and isolated somata was not detectable when impalements were made with QX-314-filled electrodes (cf., Fig. 6, *A2* and *A3*; see also Connors and Prince, 1982). These data suggest that a slow, voltage-dependent conductance resistant to QX-314 and presumably due to Ca^{2+} is present predominantly in the apical dendrites of CA1 pyramidal neurons, whereas a non-inactivating Na^+ conductance is prominent during subthreshold depolarization in the somata of the same neurons. Further support for this conclusion has been obtained recently in preliminary experiments which show that AR in dendrites is depressed significantly by Mn^{2+} (L. S. Benardo, L. M. Masukawa, and D. A. Prince, unpublished observations) and that AR in isolated somata is abolished by QX-314.

Discussion

The experiments described here extend the findings of Wong et al. (1979) and Schwartzkroin and Prince (1980) obtained from intradendritic recordings in intact hippocampal slice preparations and provide data relevant to three specific issues which are discussed below.

Apical dendrites of CA1 HPCs generate both Na^+- and Ca^{2+}-mediated spikes. Our data show that isolated apical dendritic membranes of CA1 HPCs can generate QX-314-sensitive fast spikes which presumably are mediated by Na^+ as well as slow spikes which are resistant to Na^+ channel blockade and presumably due to Ca^{2+} influx. This conclusion is supported by the results of recent experiments which show that isolated dendrites impaled in the presence of Mn^{2+} generate only fast spikes (L. S. Benardo, L. M. Masukawa, and D. A. Prince, unpublished observations). In recordings made with potassium acetate-filled electrodes, dendritic membranes generate both varieties of spikes when directly depolarized. The occurrence of spikes with inflections on their falling phases and of more discrete fast and slow action potentials of different amplitudes suggests that there are multiple sites of spike generation in the apical dendritic tree and that spike propagation is discontinuous over the dendritic membrane. Multi-notched spikes also have been seen in Purkinje cell dendrites (Llinas and Nicholson, 1971; Llinas and Sugimori, 1980b) where they have been interpreted as the result of the discontinuous propagation of Ca^{2+} spikes. In CA1 HPC dendrites, composite or fractionated spikes usually are initiated by a fast spike, suggesting that Na^+ spikes may serve as a trigger for subsequent higher threshold regenerative Ca^{2+} events. This conclusion is supported by the finding that fractionated spikes containing fast and slow components were no longer seen after exposure to QX-314; only discrete slow

spikes could be elicited under these conditions. The intermediate duration spikes which are present in intact and isolated dendrites, but not in QX-314-treated dendrites (Table I), also may be examples of potentials generated by both Na^+ and Ca^{2+} in which an inflection between the two events is not obvious. Similar potentials mediated by both Na^+ and Ca^{2+} are present in dorsal root ganglion cells (Yoshida et al., 1978) and may be generated in the somata of neurons of neocortical slices after the blockade of K^+ conductance (Connors et al., 1981).

These data do not bear on the question of whether somatic Na^+ spikes actively propagate into the apical dendrites of intact HPCs. However, the presence of fast prepotentials in intact dendrites (see also Wong et al., 1979) and their absence in recordings from isolated dendrites might suggest that they are due to passive propagation of somatic or more proximal dendritic Na^+ spikes into distal apical dendrites.

CA3 HPC dendrites also appear to be capable of generating both Na^+- and Ca^{2+}-mediated action potentials (Wong et al., 1979). However, the distribution of Na^+ spikes in HPC dendrites and somata is different from that found in Purkinje cells where Na^+ spikes are conducted passively into the dendrites but are not generated actively by dendritic membranes (Llinas and Sugimori, 1980a, b). Preliminary observations from recordings of presumed dentate granule cell dendrites suggest that their membrane properties are quite different from those of HPCs or Purkinje cells in that large amplitude slow and fast spikes have not been evoked by large depolarizing current pulses (R. Fricke and D. A. Prince, unpublished observations). Thus, it appears that different types of neurons may have fundamental differences in the properties of their dendritic membranes.

Properties of HPC somata. The contribution of Na^+ and Ca^{2+} to regenerative inward currents is clearly different in the somata and dendrites of hippocampal CA1 pyramidal cells. The finding that isolated and intact cell bodies ordinarily generate only fast action potentials, whereas isolated and intact dendrites generate both fast and slow spikes during comparable depolarizations (cf., Figs. 4*A3* and 1*B2*) and slow spikes preferentially during intense depolarization (e.g., Fig. 1*B3*) is consistent with this conclusion. Such observations support the general concept that voltage-dependent Na^+ conductances predominate in cortical somata. However, following a variety of manipulations, it also becomes possible to evoke slower, presumed Ca^{2+} spikes during intrasomatic depolarizations in CA1 HPC somata (Schwartzkroin and Slawsky, 1977; Schwartzkroin and Prince, 1980; Hotson et al., 1979; Hotson and Prince, 1980, 1981). The present experiments show that this is also the case in isolated somata (Fig. 5 and results above). Our data do not resolve the question of the site of origin of these Ca^{2+} spikes, although it is clear that a large portion of apical dendritic membrane is not required. As noted above, an "isolated" soma possesses an elaborate basilar dendritic arborization as well as the most proximal portion (~25 to 150 μm) of its apical dendrite. Any or all of these membranes might possess significant Ca^{2+} conductance and become capable of Ca^{2+} spike generation once Na^+ and/or K^+ conductances are blocked. The persistence of slow afterhyperpolarizations following trains of fast spikes in isolated somata suggests that a Ca^{2+}-activated K^+ conductance is present. This must mean that a voltage-dependent Ca^{2+} conductance (Hotson and Prince, 1980) can be activated in the soma-dendritic membrane proximal to the cut. In immature Purkinje cell somata, Na^+ conductances predominate; Ca^{2+}-mediated responses can be detected only after the main apical dendrite has begun to differentiate, strongly suggesting that Ca^{2+} conductances have only a dendritic localization (Llinas and Sugimori, 1979). The evidence on this point in HPCs remains incomplete and does not rule out a possible somatic Ca^{2+} conductance.

The ionic basis for anomalous rectification may be different in somata and dendrites of CA1 HPCs. The subthreshold behavior of HPC membranes is determined by the effects of depolarization on voltage-dependent conductances for Ca^{2+}, Na^+, and K^+. It was reported initially that, in recordings from CA1 HPC somata, an inward (depolarizing) anomalous rectification (AR) was carried by both Ca^{2+} and Na^+. When AR was depressed by either Mn^{2+} or TTX, an area of delayed rectification in the IV curve, presumably due to the activation of a K^+ conductance, was uncovered (Hotson et al., 1979). Subsequent studies have shown the presence of a subthreshold Ca^{2+} conductance in CA3 HPC somata, where AR is also prominent (Wong and Prince, 1978, 1981; Johnston et al., 1980). The results of more recent experiments suggest that Na^+ may be a more significant mediator of AR than Ca^{2+} in CA1 HPC somata (Benardo and Prince, 1982). A prominent subthreshold Na^+ conductance also has been reported recently in Purkinje cells (Llinas and Sugimori, 1980a), neocortical neurons *in vitro* (Connors et al., 1981; Prince, 1982), and HPCs (Connors and Prince, 1982).

The pattern of distribution of slow conductances which mediate AR in the somata and dendrites of CA1 HPCs appears to be similar to that discussed above for the Na^+ and Ca^{2+} conductances underlying spike generation. Specifically, although AR is present in both isolated dendrites and somata, it is greatly reduced by QX-314 in intact and isolated somata but not in isolated dendrites. This suggests that the major charge carrier for inward subthreshold current is Ca^{2+} in dendrites and Na^+ in somata. As mentioned above, this conclusion is supported by the finding that Mn^{2+} produces significant depression of AR in dendrites (L. S. Benardo, L. M. Masukawa, and D. A. Prince, unpublished observations). Thus, subthreshold dendritic excitability is due primarily to Ca^{2+} electrogenesis, although we cannot exclude a minor contribution by subthreshold Na^+ conductances. The presence of both Na^+ and Ca^{2+} components of AR during depolarization through an intrasomatic electrode (Hotson et al., 1979) presumably is due to local (somatic) activation of gNa^+ and the spread of applied current into apical dendritic regions where subthreshold gCa^{2+} is activated.

One issue in the interpretation of the electrophysiological results obtained from brain slices maintained *in vitro* has been the potential contribution of injury or other iatrogenic factors. The same question certainly can be raised in the present experiments where even more radical dissection has been used. In fact, it is remarkable

that some isolated somata and dendrites appear to "seal" their severed processes and maintain their normal membrane potentials, input resistances, spike heights, and a variety of ionic conductances for hours after such treatment, suggesting that injury has not made a major contribution to the data obtained in these studies. We cannot, however, rule out effects of injury per se on a specific conductance. For example, it is possible that somatic Ca^{2+} conductance is fragile and easily suppressed as has been reported in motoneurons (Schwindt and Crill, 1980). This could influence conclusions regarding the relative roles of subthreshold Ca^{2+} and Na^{+} conductances in mediating depolarizing AR in somata versus dendrites.

The electroresponsive properties of dendritic membranes in CA1 HPCs are quite complex and include both subthreshold conductances as well as those leading to regenerative events. Our results suggest that action potentials mediated by Ca^{2+} and Na^{+}, a voltage-dependent slow subthreshold Ca^{2+} conductance, and a Ca^{2+}-activated K^{+} conductance originate in the dendrites of CA1 pyramidal cells. In addition, voltage-dependent conductances also occur in response to hyperpolarizing current. These active membrane events must influence dendritic responses to synaptic activation significantly. Attempts to describe the integrative functions of these neurons must take into account the variety of conductances which are activated nonuniformly in somata and dendrites by changes in membrane potential. Such responses, in addition to the passive membrane properties and electrotonic structure of these elements, must have a powerful effect on signal processing in the hippocampus.

References

Benardo, L. S., and D. A. Prince (1982) Ionic mechanisms of cholinergic excitation in mammalian hippocampal pyramidal cells. Brain Res., *249:* 333–334.

Brown, T. H., R. A. Fricke, and D. H. Perkel (1981) Passive electrical constants in three classes of hippocampal neurons. J. Neurophysiol. *46:* 812–827.

Connors, B. W., and D. A. Prince (1982) Effects of local anesthetic QX-314 on the membrane properties of hippocampal pyramidal neurons. J. Pharmacol. Exp. Ther. *200:* 476–481.

Connors, B. W., M. J. Gutnick, and D. A. Prince (1981) Electrophysiological properties of neocortical neurons maintained *in vitro*. Soc. Neurosci. Abstr. *7:* 593.

Hagiwara, S., J. Fukada, and D. C. Eaton (1974) Membrane currents carried by Ca, Sr, and Ba in barnacle muscle fiber during voltage clamp. J. Gen. Physiol. *63:* 564–578.

Hotson, J. R., and D. A. Prince (1980) A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. J. Neurophysiol. *43:* 409–419.

Hotson, J. R., and D. A. Prince (1981) Penicillin- and barium-induced epileptiform bursting in hippocampal neurons: Actions on Ca^{++} and K^{+} potentials. Ann. Neurol. *10:* 11–17.

Hotson, J. R., D. A. Prince, and P. A. Schwartzkroin (1979) Anomalous inward rectification in hippocampal neurons. J. Neurophysiol. *42:* 889–895.

Johnston, D., J. J. Hablitz, and W. A. Wilson (1980) Voltage clamp discloses slow inward current in hippocampal burst-firing neurons. Nature *286:* 391–393.

Llinas, R. (1975) Electroresponsive properties of dendrites in central neurones. Adv. Neurol. *12:* 1–13.

Llinas, R., and R. Hess (1976) Tetrodotoxin-resistant dendritic spikes in avian Purkinje cells. Proc. Natl. Acad. Sci. U. S. A. *73:* 2520–2523.

Llinas, R., and C. Nicholson (1971) Electrophysiological properties of dendrites and somata in alligator Purkinje cells. J. Neurophysiol. *34:* 532–551.

Llinas, R., and M. Sugimori (1979) Calcium conductances in Purkinje cell dendrites: Their role in development and integration. Prog. Brain Res. *51:* 323–334.

Llinas, R., and M. Sugimori (1980a) Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. J. Physiol. (Lond.) *305:* 171–195.

Llinas, R., and M. Sugimori (1980b) Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. J. Physiol. (Lond.) *305:* 197–213.

Llinas, R., C. Nicholson, J. Freeman, and D. E. Hillman (1968) Dendritic spikes and their inhibition in alligator Purkinje cells. Science *160:* 1132–1135.

Masubawa, L. M., L. S. Bernardo, and D. A. Prince (1982) Variations in electophysiological properties of hippocampal neurons in different subfield. Brain Res. *242:* 341–344.

Nelson, P. G., and K. Frank (1967) Anomalous rectification in cat spinal motorneurons and effect of polarizing currents on excitatory postsynaptic potential. J. Neurophysiol. *30:* 1097–1113.

Prince, D. A. (1982) Epileptogenesis in hippocampal and neocortical neurons. In *Physiology and Pharmacology of Epileptogenic Phenomena*, M. R. Klee, H. D. Lux, and E. J. Speckmann, eds., pp. 151–162, Raven Press, New York.

Purpura, D. P. (1967) Comparative physiology of dendrites. In *The Neurosciences*, G. C. Quarton, T. Melchuk, and F. O. Schmidt, eds., pp. 372–393, Rockefeller University Press, New York.

Purpura, D. P. (1969) Stability and seizure susceptibility in immature brain, In Basic Mechanisms of the Epilepsies, H. H. Jasper, A. A. Ward, Jr., and A. Pope, eds., pp. 481–505, Little, Brown and Co., Boston.

Purpura, D. P., and J. G. McMurtry (1965) Intracellular activities and evoked potential changes during polarization of motor cortex. J. Neurophysiol. *28:* 166–185.

Purpura, D. P., and R. J. Shofer (1964) Cortical intracellular potentials during augmenting and recruiting responses. I. Effects of injecting hyperpolarizing currents on evoked membrane potential changes. J. Neurophysiol. *27:* 117–132.

Purpura, D. P., R. J. Shofer, and T. Scarff (1965) Properties of synaptic activities and spike potentials of neurons in immature neocortex. J. Neurophysiol. *28:* 925–942.

Purpura, D. P., J. G. McMurtry, C. F. Leonard, and A. Malliani (1966) Evidence for dendritic origin of spikes without depolarizing prepotentials in hippocampal neurons during and after seizure. J. Neurophysiol. *29:* 954–979.

Schwartzkroin, P. A. (1975) Characteristics of CA1 neurons recorded intracellularly in the hippocampal *in vitro* slice preparation. Brain Res. *85:* 423–436.

Schwartzkroin, P. A. (1977) Further characteristics of hippocampal CA1 cells *in vitro*. Brain Res. *128:* 53–68.

Schwartzkroin, P. A., and L. H. Mathers (1978) Physiological and morphological identification of a nonpyramidal hippocampal cell type. Brain Res. *157:* 1–10.

Schwartzkroin, P. A., and D. A. Prince (1978) Cellular and field potential properties of epileptogenic hippocampal slices. Brain Res. *147:* 117–130.

Schwartzkroin, P. A., and D. A. Prince (1980) Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. Brain Res. *183:* 61–76.

Schwartzkroin, P. A., and M. Slawsky (1977) Probable calcium spikes in hippocampal neurons. Brain Res. *135:* 157–161.

Schwindt, P. C., and W. E. Crill (1980) Properties of a persistent inward current in normal and TEA-injected motoneurones. J. Neurophysiol. *43:* 1700–1724.

Spencer, W. A., and E. R. Kandel (1961) Electrophysiology of hippocampal neurons. IV. Fast prepotentials. J. Neurophysiol. *24:* 272–285.

Turner, D. A., and P. A. Schwartzkroin (1980) Steady-state

electrotonic analysis of intracellularly stained hippocampal neurons. J. Neurophysiol. *44:* 184–199.

Westrum, L. E., L. E. White, and A. A. Ward, Jr. (1964) Morphology of the experimental epileptic focus. J. Neurosurg. *21:* 1033–1046.

Wong, R. K. S., and D. A. Prince (1977) Burst generation and calcium mediated spikes in hippocampal CA3 neurons. Soc. Neurosci. Abstr. *3:* 148.

Wong, R. K. S., and D. A. Prince (1978) Participation of calcium spikes during intrinsic burst firing in hippocampal neurons. Brain Res. *159:* 385–390.

Wong, R. K. S., and D. A. Prince (1979) Dendritic mechanisms underlying penicillin induced epileptiform activity. Science *204:* 1228–1231.

Wong, R. K. S., and D. A. Prince (1981) Afterpotential generation in hippocampal pyramidal cells. J. Neurophysiol. *45:* 86–97.

Wong, R. K. S., D. A. Prince, and A. I. Basbaum (1979) Intradendritic recordings from hippocampal neurons. Proc. Natl. Acad. Sci. U. S. A. *76:* 986–990.

Yamamoto, C. (1972) Activation of hippocampal neurons by mossy fiber stimulation in thin brain sections *in vitro*. Exp. Brain Res. *14:* 423–435.

Yoshida, S., Y. Matsuda, and A. Samejima (1978) Tetrodotoxin-resistant sodium and calcium components of action potentials in dorsal root ganglion cells of the adult mouse. J. Neurophysiol. *41:* 1096–1106.