

# Linear Summation of Excitatory Inputs by CA1 Pyramidal Neurons

Sydney Cash and Rafael Yuste  
Department of Biological Sciences  
Columbia University  
New York, New York 10027

## Summary

A fundamental problem in neurobiology is understanding the arithmetic that dendrites use to integrate inputs. The impact of dendritic morphology and active conductances on input summation is still unknown. To study this, we use glutamate iontophoresis and synaptic stimulation to position pairs of excitatory inputs throughout the apical, oblique, and basal dendrites of CA1 pyramidal neurons in rat hippocampal slices. Under a variety of stimulation regimes, we find a linear summation of most input combinations that is implemented by a surprising balance of boosting and shunting mechanisms. Active conductances in dendrites paradoxically serve to make summation linear. This “active linearity” can reconcile predictions from cable theory with the observed linear summation *in vivo* and suggests that a simple arithmetic is used by apparently complex dendritic trees.

## Introduction

One essential function of mammalian neurons is to integrate the thousands of inputs that arrive on their dendritic trees. How neurons accomplish this basic arithmetic task still remains unknown. Two interrelated features, the geometrically intricate dendritic arborization (Ramón y Cajal, 1904) and the rich variety of voltage-sensitive channels distributed heterogeneously throughout the cell (Llinás, 1988; Johnston et al., 1996; Yuste and Tank, 1996), are expected to greatly influence input integration. Theoretical analyses of input summation began with the assumption that dendrites can be modeled as passive cables (Jack et al., 1975; Rall, 1995). Cable theory predicts that electrically isolated synaptic inputs sum linearly, while ones that are electrically close are attenuated due to reduction in the ionic driving force or current shunting caused by a transient decrease of dendritic input resistance (Rall, 1964). This simple concept gives a powerful *raison d'être* to the dendritic tree, since dendritic branches would then serve to isolate inputs from one another. Unfortunately, experimental probing of these ideas has been surprisingly scant. Analysis of synaptic potentials from motoneurons *in vivo* revealed summed potentials that were less than expected (Burke, 1967; Kuno and Miyahara, 1969). This discrepancy was ascribed to sublinear interactions between nearby synapses. In CA1 hippocampal pyramidal neurons *in vitro*, however, inputs summated linearly (Langmoen and Andersen, 1983). Also, iontophoresis on dendrites of spinal cord motoneurons *in vitro* showed linear integration (Skydsgaard and Hounsgaard, 1994). Finally, linear summation of inputs has been found

throughout the visual system in a long tradition of psychophysical and electrophysiological experiments *in vivo* (Jagadeesh et al., 1991; reviewed by Wandell, 1995). These *in vivo* results are in apparent contradiction with the predictions from cable theory, because large inputs fail to show significant shunting (Jagadeesh et al., 1997). In all of these experiments, however, neither the exact input position nor the dendrite architecture was determined, so the specific effects of morphology on summation were not explored.

To study input integration by dendrites, the ideal experiment is to stimulate individual synaptic inputs at known positions of the dendritic tree. Unfortunately, although electrical stimulation reliably triggers excitatory postsynaptic potentials (EPSPs), it is difficult to determine exactly where these inputs impinge upon the dendrites. In contrast, microiontophoresis of glutamate can be used to precisely place a realistic excitatory input at any desired location on the dendritic arbor. Furthermore, iontophoresis is spatially restricted, is not affected by pharmacological manipulations that would alter synaptic release, and can easily be adjusted in amplitude.

In recent work using glutamate iontophoresis on cultured hippocampal neurons, we found linear summation of excitatory inputs that was independent of dendritic morphology and, surprisingly, arose from a balanced activation of NMDA and  $K^+$  channels (Cash and Yuste, 1998). In the same study, we failed to find evidence for the effect of branching on summation predicted by Rall (1964). Nevertheless, while cultured neurons provide an excellent model system, they may have different ion channels and receptors than neurons *in vivo*, and it is likely that their physical or electrotonic structure is also different. To address how dendritic morphology and active conductances influence synaptic integration in more realistic neurons, we have now used both glutamate microiontophoresis and EPSP stimulation of hippocampal CA1 pyramidal neurons in rat brain slices and, in addition, have explored regimes of stimulation in which hundreds of synapses are excited to mimic the activity state of neurons *in vivo*. We find that linear summation is the rule in CA1 pyramidal neurons in slices and that linearity is achieved by a set of different types of cellular mechanisms involving active conductances that counteract the local shunting. These mechanisms provide a linearizing function for active dendrites and can resolve the discrepancy between the theoretically expected sublinear interaction between inputs and the linear summation found *in vivo* in many systems.

## Results

### Glutamate Iontophoresis Produces Spatially Restricted Depolarizations that Resemble EPSPs

We first explored whether iontophoresis of glutamate could reliably be used in brain slices to mimic EPSPs at particular dendritic locations (Figure 1). By filling CA1 pyramidal neurons with the fluorescent marker rhodamine dextran through the recording pipette, the dendritic

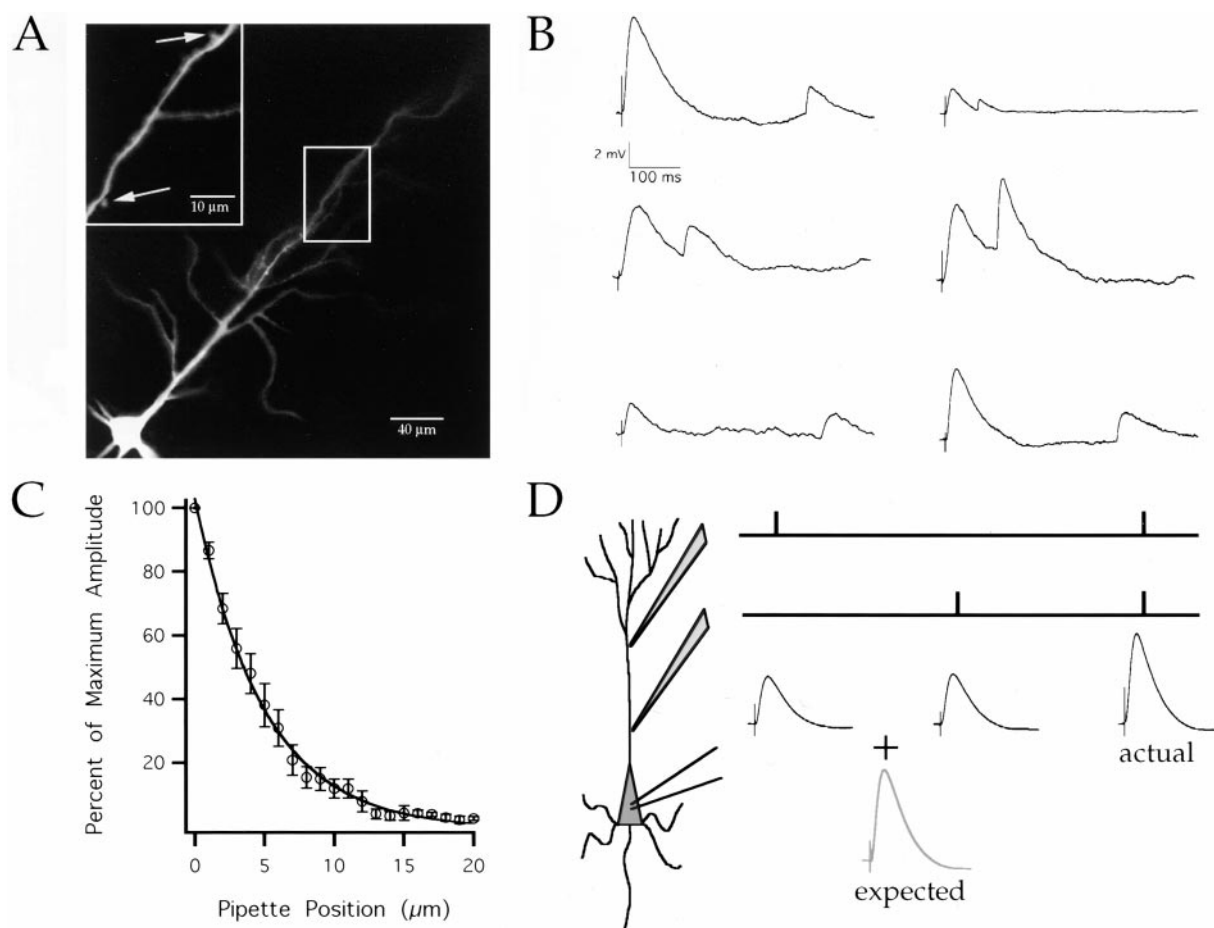


Figure 1. Glutamate Iontophoresis Produces Spatially Restricted Depolarizations that Resemble EPSPs

(A) Photomicrograph of a living CA1 pyramidal neuron filled through a whole-cell patch pipette with rhodamine dextran. Inset shows the positions of the tips (arrows) of two microiontophoresis pipettes near the distal apical dendrite. The entire dendritic tree can be thus imaged and iontophoresis pipettes precisely positioned at specific locations throughout the tree.

(B) Iontophoretic events resemble spontaneous EPSPs. Examples from recordings in which the first depolarization is an iontophoretic event, and secondary depolarizations are spontaneous EPSPs. Note how the kinetics of both types of events are very similar.

(C) Iontophoresis is highly localized. An iontophoresis pipette was positioned near the apical dendrite (as in [A]) to achieve maximum depolarization and then elevated away from the dendrite in 1 μm increments. The peak amplitude dropped e-fold in 4.9 μm; solid line indicates the best single exponential fit.

(D) Protocol for testing summation. Stimulation was delivered first from each pipette individually and then from both pipettes simultaneously. The combined event was compared with the expected, linear sum (gray line) of the two individual events.

tree could be visualized and the microiontophoresis pipette positioned within microns of a particular dendrite (Figure 1A). Neurons were recorded at a resting potential of  $-60$  mV and had input resistances of  $164 \pm 11$  MΩ, with time constants of  $22 \pm 2$  ms ( $n = 20$ ). Depolarizations resulting from glutamate iontophoresis and EPSPs were very similar in their kinetic properties; the 10/90 rate of rise for iontophoresis was  $0.53 \pm 0.04$  mV/ms versus  $0.63 \pm 0.04$  mV/ms for EPSPs, and the decay time constant for iontophoresis was  $39.6 \pm 0.3$  ms versus  $42.9 \pm 0.2$  ms for EPSPs ( $n = 5$ ; Figure 1B). We also found that glutamate iontophoresis application was spatially very restricted, with a 5 μm movement of the pipette tip resulting in an e-fold change in amplitude (Figure 1C). Finally, we tested whether subsequent iontophoresis produced reliable responses. Indeed, with our experimental protocol ( $>5$  s interval between stimuli), repeated single trials produced reproducible responses ( $100\% \pm 3\%$ ,  $n = 14$ ). Microiontophoresis of

glutamate, therefore, has the appropriate spatial resolution and reproducibility in brain slices to be used to probe the properties of the dendritic tree.

#### Summation of Iontophoretic Inputs and EPSPs Is Largely Linear and Depends on Input Position

We first determined how two inputs on different positions of the dendritic tree summed using simultaneous iontophoresis. The basic experiment was simple (Figure 1D). While recording from the soma, iontophoresis pipettes were positioned systematically on the apical, oblique, and basal dendritic arbor. The iontophoresis from each pipette was adjusted to evoke a subthreshold depolarization, even when both were activated simultaneously. The depolarization caused by each input was measured separately, and then both were tested simultaneously. The combined response was then compared with the expected, linear sum of the individual events.

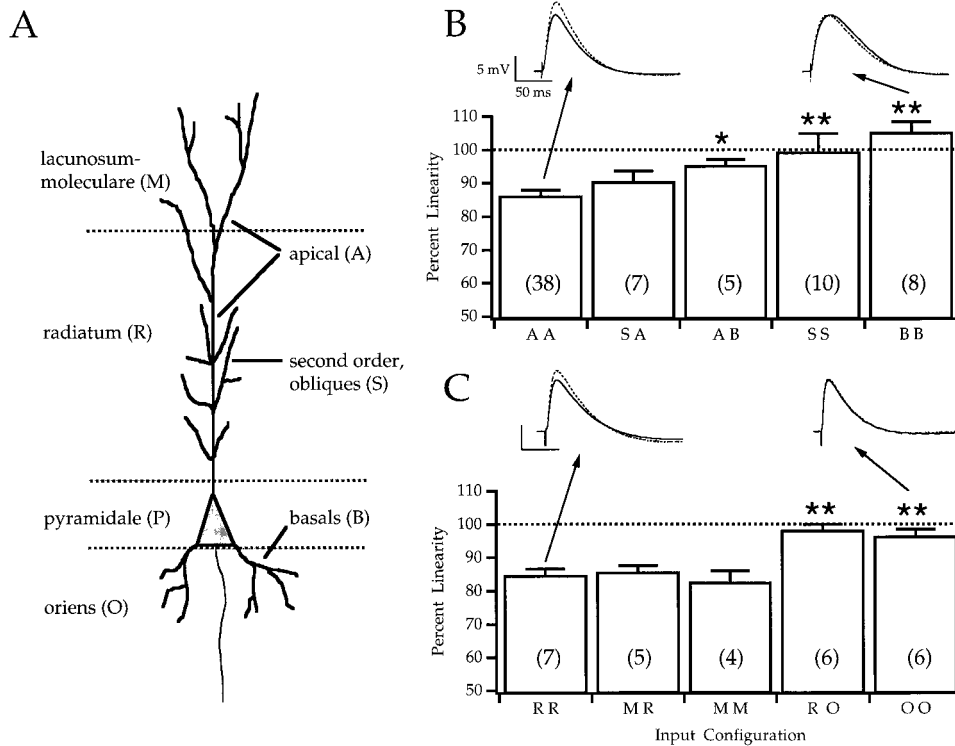


Figure 2. Summation Depends on Input Position

(A) Schematic diagram of a CA1 pyramidal neuron showing dendritic locations and layers tested.

(B) Histogram of the linearity of the summed iontophoresis responses for different input configurations. Linearity is expressed as the ratio of the actual peak amplitude of the combined event to the expected peak amplitude, calculated by adding the two separate events. When excitatory inputs were both on the apical dendrite (AA) or on the apical and an oblique (AO), summation was sublinear. Inputs on an oblique dendrite (SS) or on the apical and a basal dendrite (AB) is linear. Summation is linear when both inputs are on the same basal dendrite (BB). Trace insets show examples. For all figures, solid line is observed sum and dotted line is expected sum.

(C) Synaptic stimulation shows a similar dependency on input location. Integration is sublinear when inputs are in the radiatum (RR) or lacunosum-moleculare (MR, MM) layers but is linear when the inputs are in the radiatum and oriens (RO) or oriens alone (OO). Trace insets show examples. Scale bar, 5 mV, 50 ms; asterisk,  $p < 0.05$ ; double asterisk,  $p < 0.01$  versus AA or RR.

When both inputs were on the apical dendrite, summation was slightly sublinear, i.e., the combined event was smaller than the arithmetic sum of the individual events ( $86\% \pm 2\%$  of expected,  $n = 38$ ; Figures 2A and 2B). When the inputs were on an oblique and an apical dendrite distal to the first branch point, summation was also sublinear ( $90\% \pm 3\%$ ,  $n = 7$ ). When both inputs were on the oblique dendrites, summation was linear ( $99\% \pm 5\%$ ,  $n = 10$ ). If one input was on a basal dendrite and the other on the apical, summation was also effectively linear ( $95\% \pm 2\%$ ,  $n = 5$ ). Finally, when both inputs were on the same basal dendrite, integration was also linear ( $105\% \pm 3\%$ ,  $n = 8$ ).

These iontophoresis results were corroborated by extracellular stimulation in the stratum oriens, radiatum, and lacunosum-moleculare, exciting inputs onto the basal dendrites, apical tree, and distal apical tree, respectively (Figures 2A and 2C). As described above, EPSPs caused by each input were measured separately, then elicited simultaneously and compared with their expected sum. We assumed that each stimulating electrode excited an independent axonal pathway, because we never detected paired pulse interactions between the pipettes. When both stimulating electrodes were in the upper portion of the radiatum, a situation similar

to iontophoretic application along the apical and/or oblique dendrites, integration was also sublinear ( $84\% \pm 2\%$ ,  $n = 7$ ). This was also true if the inputs were in the lacunosum-moleculare ( $83\% \pm 3\%$ ,  $n = 4$ ) or in both radiatum and lacunosum-moleculare ( $86\% \pm 2\%$ ,  $n = 5$ ). In contrast, if both stimulation electrodes were in the oriens, or if one was in the oriens and one in the radiatum, then integration was linear ( $97\% \pm 2\%$  and  $98\% \pm 2\%$  respectively,  $n = 6$ ). These positions correspond to iontophoresis in the basal dendrites or to one input on a basal and one in the apical, respectively.

The excellent agreement between iontophoresis and synaptic stimulation results strongly suggests that both methods reveal the same sensitivity of summation to input position; while signals from most input positions sum linearly, inputs on the apical dendrite appear to slightly diminish their joint effect.

#### An $I_A$ Potassium Conductance Mediates Sublinear Summation on the Apical Dendrite

To understand how active conductances influence input summation in different dendritic positions, we first focused on the sublinear summation in the apical dendrite and inquired whether it depended on the amplitude of the depolarization. Indeed, we found that iontophoretic

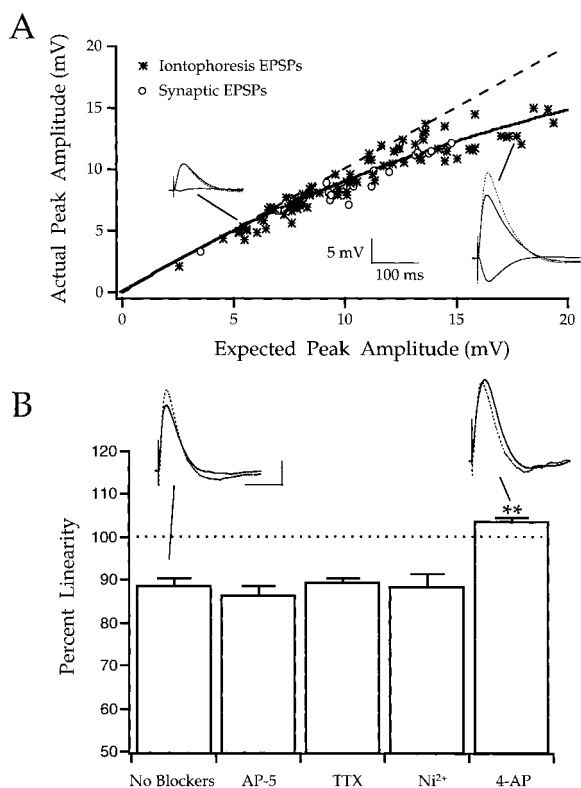


Figure 3. Mechanisms of Summation on Apical Dendrites

(A) Two iontophoretic inputs on the apical dendrite (stars) or extracellular stimulation in the stratum radiatum (open circles) add linearly at small amplitudes but sublinearly at larger amplitudes approaching threshold. Dotted line shows expected linear summation, and solid line is a single exponential fit to the iontophoresis data (147 experiments in 67 cells;  $p < 0.0001$ ). Insets, individual examples. Solid downward traces represent subtraction of expected from actual events and is included to demonstrate the sublinearity observed with large amplitude events.

(B) Sublinear integration is mediated by  $I_A K^+$  channels. Bath application of the NMDA receptor blocker AP-5 (100  $\mu$ M),  $Na^+$  channel blocker TTX (5  $\mu$ M), and  $Ca^{2+}$  channel blocker  $Ni^{2+}$  (1 mM) did not alter the sublinearity. Application of the  $I_A K^+$  channel blocker 4-AP (3 mM), however, removed the sublinearity of integration ( $p < 0.0003$ ,  $n = 5$ ). Insets show the results from the same cell before and after the application of 4-AP (dotted line, expected; solid line, measured result).

potentials on the apical dendrite showed an increasingly sublinear summation with increasing amplitude (Figure 3A). For example, events of expected peak amplitude  $<10$  mV added only slightly sublinearly ( $92\% \pm 2\%$ ,  $n = 15$  cells). Events between 10 and 15 mV added  $88\% \pm 2\%$  ( $n = 32$ ) of expected, while inputs  $>15$  mV and very close to threshold only summed to  $78\% \pm 4\%$  of expected ( $n = 6$ ). This amplitude-dependent sublinearity was also evident in a comparison of the integrals of the expected and actual potentials; smaller events added almost linearly ( $96\% \pm 2\%$ ), larger events more sublinearly ( $91\% \pm 2\%$ ), and near threshold events even more sublinearly ( $83\% \pm 7\%$ ). This sublinearity was not due to systematic changes in the glutamate delivery or receptor sensitivity, because repeated single trials produced reproducible responses (data not shown). Again, these results were complemented by synaptic stimulation experiments in which two stimulating electrodes

were positioned in the stratum radiatum. As with iontophoresis, small EPSPs ( $<10$  mV) added almost linearly ( $95\% \pm 3\%$ ,  $n = 5$ ) while larger EPSPs ( $>10$  mV) added sublinearly ( $85\% \pm 2\%$ ,  $n = 7$ ; Figure 3A, circles).

To better understand the mechanisms underlying this amplitude-dependent sublinearity in apical dendrites, we characterized the summation of large amplitude iontophoretic inputs ( $>10$  mV expected) in the presence of various pharmacological blockers (Figure 3B). We first assessed the role of the NMDA receptor in summation, since its activation can produce boosting of synaptic inputs (Thomson et al., 1988), and then studied the contribution of the known dendritic  $Na^+$ ,  $Ca^{2+}$ , and  $K^+$  conductances (Johnston et al., 1996). We found that summation was not significantly affected when NMDA receptors were blocked with AP-5 (100  $\mu$ M;  $86\% \pm 2\%$ ,  $n = 5$ ). The  $Na^+$  channel blocker tetrodotoxin (TTX) abolished all action potentials but produced no significant difference in summation compared with controls (5  $\mu$ M;  $89\% \pm 1\%$ ,  $n = 5$ ). Application of the  $Ca^{2+}$  channel blocker  $Ni^{2+}$  also did not produce a statistically significant difference in the sublinearity (1 mM;  $88\% \pm 3\%$ ,  $n = 5$ ). In contrast, blocking  $I_A K^+$  conductances with 4-amino-pyridine (4-AP; 3 mM) abolished the sublinearity ( $103\% \pm 1\%$  in 4-AP versus  $88\% \pm 2\%$  in control,  $n = 5$ ,  $p < 0.0003$ , ANOVA). We wondered if the effect of 4-AP was due to changes in the passive properties of the apical dendrite that could affect filtering of the inputs. 4-AP did not, however, cause significant changes in kinetic parameters of the iontophoretic potentials, indicating that the filtering properties of the dendrite were not substantially altered by 4-AP application (peak amplitude before 4-AP,  $12.4 \pm 0.8$  mV versus  $11.4 \pm 0.8$  in 4-AP; 10%/90% rate of rise,  $0.56 \pm 0.06$  mV/ms before 4-AP versus  $0.41 \pm 0.05$  in 4-AP; decays,  $30 \pm 3$  ms before 4-AP versus  $37 \pm 4$  in 4-AP; ANOVA,  $p > 0.05$ ,  $n = 5$ ). In addition, during sublinear summation, the peak of the observed potential did not shift significantly ( $-0.3 \pm 0.4$  ms,  $n = 39$ ), as would have been expected for a purely passive mechanism (G. Major, personal communication). Finally, to test the effect of the membrane potential on the sublinearity, we hyperpolarized the neuron to  $-80$  mV and measured summation of iontophoretic events in the distal apical dendrite, finding a summation of  $82\% \pm 4\%$  ( $n = 6$ ), similar to control ( $86 \pm 2$ ,  $n = 38$ ). The lack of effect of hyperpolarization is probably due to the distant source of the input. Taken together, these results indicate that the sublinear summation of large inputs in the apical dendrite is mediated by activation of a dendritic 4-AP-sensitive  $K^+$  channel, most likely  $I_A$  (Hoffman et al., 1997).

#### The Sublinearity in the Apical Dendrite Has a Laminar Dependency and Is Developmentally Regulated

The distribution of  $I_A K^+$  channels along the apical dendrite in CA1 neurons increases in density with further distance from the soma (Hoffman et al., 1997). We therefore wondered if the sublinearity revealed in the apical dendrite also had a similar spatial profile. Indeed, we found that events that were closer to the soma summed linearly, while distal inputs summed sublinearly, approaching in some cases a 30% reduction from the expected effect (Figure 4A;  $R = -0.75$ ,  $p < 0.0001$ , ANOVA).

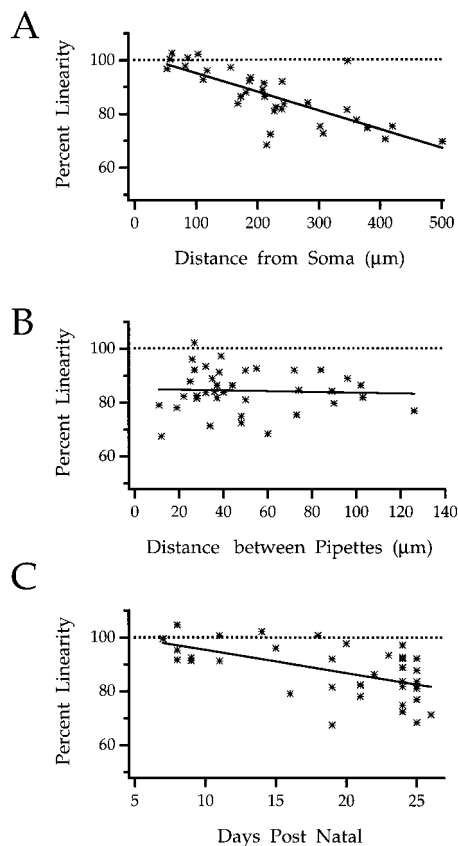


Figure 4. The Sublinearity on the Apical Dendrite Depends on Input Location and on Age but Not on Interpipette Distance

(A) The extent of sublinear summation of large (>10 mV expected) excitatory events on the apical dendrite is dependent on the distance of those inputs from the soma. More distal inputs sum more sublinearly. Best linear fit is shown by the solid line ( $R = -0.75$ ,  $p < 0.0001$ , ANOVA).

(B) Summation of iontophoretic stimuli is not affected by the distance separating the pipettes. All inputs were on the apical dendrite, 125 to 225  $\mu\text{m}$  from the soma, and were >10 mV. Line is best linear fit ( $R = -0.013$ ,  $p > 0.7$ , ANOVA).

(C) Summation of iontophoretic stimuli on the apical dendrite depends on age. Summation in slices from younger animals was more linear ( $R = -0.51$ ,  $p < 0.0005$ , ANOVA).

Is the sublinearity in the apical tree affected by the distance between inputs? As predicted by cable properties (Rall, 1964), a strong distance dependence would be expected for interacting inputs. Iontophoresis pipettes were again positioned along the apical dendrite, with the more proximal input at least 135  $\mu\text{m}$  from the soma. Interestingly, no significant correlation between intrapipette distance and summation was observed over the range of  $\sim 10$  to 125  $\mu\text{m}$  interpipette distance (Figure 4B), indicating that distance between inputs does not significantly affect spatial summation of inputs.

The sublinear, position- and distance-dependent integration seen in CA1 pyramidal neurons apparently contradicts our previous results using cultured hippocampal neurons (Cash and Yuste, 1998). In those experiments, integration was linear due to a balanced activation of NMDA and  $\text{K}^+$  channels, with no significant dependence on input position. The density and location of receptors

and voltage-dependent channels, however, probably changes during development, producing changes in integration during neuronal maturation. We therefore carried out a developmental study of the sublinearity of apical dendritic input summation. We found a significant inverse correlation of age and linearity ( $R = -0.5$ ,  $p < 0.0005$ ; Figure 4C). Neurons from younger animals showed linear integration ( $98\% \pm 3\%$ ,  $n = 4$ ; P7–P8) similar to that of cultured neurons. In fact, as in cultured neurons, blockade of NMDA receptors with AP-5 (100  $\mu\text{M}$ ) also revealed a sublinear integration in neurons from younger animals ( $86\% \pm 2\%$ ,  $n = 6$ ,  $p < 0.001$ , ANOVA; P7–8). This is consistent with a progressively smaller NMDA component in EPSPs during development (Wu et al., 1996; Malenka and Nicoll, 1997), as well as with developmental increases in dendritic  $\text{I}_A$  current (D. Johnston, personal communication).

#### Mechanisms of Summation in Oblique Dendrites

After analyzing the apical dendrites, we focused on the linear summation detected on the oblique dendrites. We defined oblique dendrites as any secondary branch of the apical dendrite that emerged within 200  $\mu\text{m}$  of the soma. Oblique dendrites in pyramidal neurons have distinct morphometric characteristics that set them apart from the rest of the apical dendritic tree (Larkman, 1991), and in agreement with this, we found in our experiments that while summation of inputs in the apical dendrite was sublinear, integration of two inputs in an oblique dendrite was significantly different and linear (Figure 2B).

To dissect the mechanisms underlying the linear summation in oblique dendrites, we again tested whether it depended on the amplitude of the iontophoretic inputs. Over the range tested (4–18 mV, combined depolarizations), summation of two inputs onto the same oblique dendrites was linear (Figure 5A). As with the apical dendrite, we tested whether active conductances were involved in maintaining this linearity by blocking the function of particular populations of channels. As opposed to the apical dendrite, we found that AP-5, TTX, and  $\text{Ni}^{2+}$  did produce a significant and reversible reduction in the combined effect of two inputs, resulting in a small but clear sublinear summation (Figure 5B). This indicates that the oblique dendrites have a complement of NMDA,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$  channels that boost excitatory inputs. To test if these active boosting effects had a heterogeneous spatial distribution within the oblique dendrites similar to the sublinear effects on the apical dendrite, we analyzed our results as a function of distance from the soma (Figure 5C). We found no significant correlation between the linear summation and the distance along the oblique dendrites, which suggests that the combined boosting effect of NMDA,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$  channels is spatially homogeneous within the oblique dendrites. We also tested if this boosting had a local spatial extent by analyzing the combined depolarization as a function of the distance between the pipettes (Figure 5D). Within the ranges explored (5 to 38  $\mu\text{m}$ ), we could not detect a significant deviation from linearity. Inputs on the oblique dendrite are therefore boosted by NMDA,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$  conductances, but they display a linear summation that results from the balance between these boosting mechanisms and an underlying shunting effect.

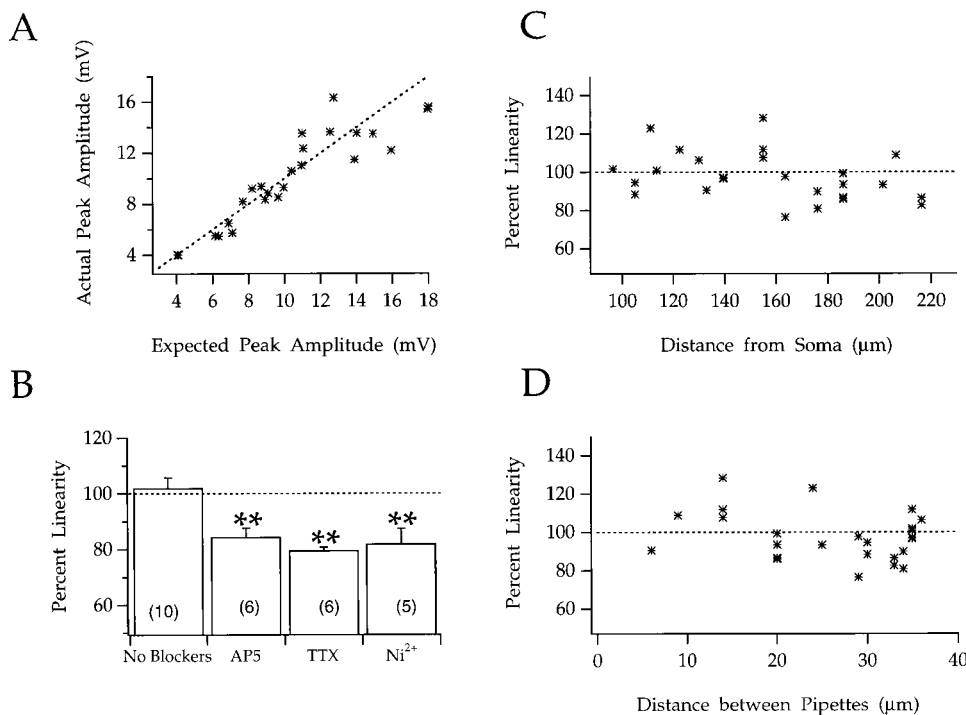


Figure 5. Mechanisms of Summation on Oblique Dendrites

(A) Two iontophoresis inputs on the same oblique dendrite add linearly at both small and large amplitudes (25 experiments; 14 cells). Dotted line shows expected linear summation.

(B) Effect of NMDA, Na<sup>+</sup>, and Ca<sup>2+</sup> channels on summation of inputs in oblique dendrites. Bath application of the NMDA receptor blocker AP-5 (100 μM), Na<sup>+</sup> channel blocker TTX (5 μM), and Ca<sup>2+</sup> channel blocker Ni<sup>2+</sup> (1 mM) significantly blocked the linearity (*p* < 0.01, ANOVA).

(C) Summation of large (8–15 mV expected) excitatory events on the oblique dendrite is not dependent on the distance of those inputs from the soma.

(D) Summation of iontophoretic stimuli on oblique dendrites is not affected by the distance separating the pipettes.

### Mechanisms of Summation in Basal Dendrites

We then focused on understanding the mechanisms underlying the summation of inputs in the basal dendritic tree by studying its amplitude and distance dependency and the effects of channel blockers. As in the oblique dendrites, we detected no significant deviation of linearity as a function of the amplitude of the iontophoretic inputs onto the same basal dendrite (Figure 6A, crosses). This lack of effect of the input amplitude was also found with EPSPs elicited with two stimulating electrodes in the stratum oriens (Figure 6A, circles). Similar to the oblique dendrites, AP-5 and TTX significantly reduced the effect of the combined iontophoresis, producing a sublinear sum (Figure 6B). The Ca<sup>2+</sup> channel blocker Ni<sup>2+</sup>, however, did not have any effect on the combined depolarization. This boosting also did not change as a function of distance from the soma (Figure 6C) or as a function of distance between the pipettes (Figure 6D). These results suggest that inputs on basal dendrites are also boosted but that Ca<sup>2+</sup> channels do not significantly contribute to the enhancement, which is carried out by NMDA receptors and Na<sup>+</sup> channels.

### A Local Sublinearity Is Present in Basal Dendrites

We wondered what were the mechanisms underlying the sublinearity revealed in basal dendrites when NMDA receptors or Na<sup>+</sup> channels were blocked. In our previous work with cultured hippocampal neurons, we uncovered

a balance of NMDA and I<sub>A</sub> K<sup>+</sup> channels that resulted in linear summation, so we therefore tested if the sublinearity in the basal dendrites in CA1 slices after the blockade of NMDA receptors was also due to I<sub>A</sub> K<sup>+</sup> channels. During application of AP-5, 4-AP did not have any effect on the sublinearity, suggesting that it was due to a different mechanism (82% ± 2% for AP-5 [*n* = 8] versus 89 ± 2 for AP-5 and 4-AP [*n* = 5]).

To explore if this sublinearity in the presence of AP-5 or TTX could spread through the dendritic tree, we positioned two inputs in the same branch of a basal dendrite and then moved one of the iontophoretic inputs to a second branch (Figure 7). This experiment thus directly investigated whether summation of inputs coming into the same dendritic branch followed the same rules as inputs located in two different branches. In the presence of either AP-5 or TTX, when two inputs were located on the same branch, summation was sublinear (79% ± 2%; Figure 7A), but the sublinearity was eliminated when the second pipette was moved to a neighboring branch (100% ± 5%, *n* = 3; Figure 7B). This result indicates that a significant sublinear summation can occur when inputs are located on the same branch but do not spread to another branch. Under normal circumstances, however, this local sublinearity is opposed by the boosting mediated by NMDA and Na<sup>+</sup> channels. The fact that the peak of the combined depolarization is delayed compared with the peak of the expected sum (Figure 7A, inset), strongly suggests that this local sublinearity may

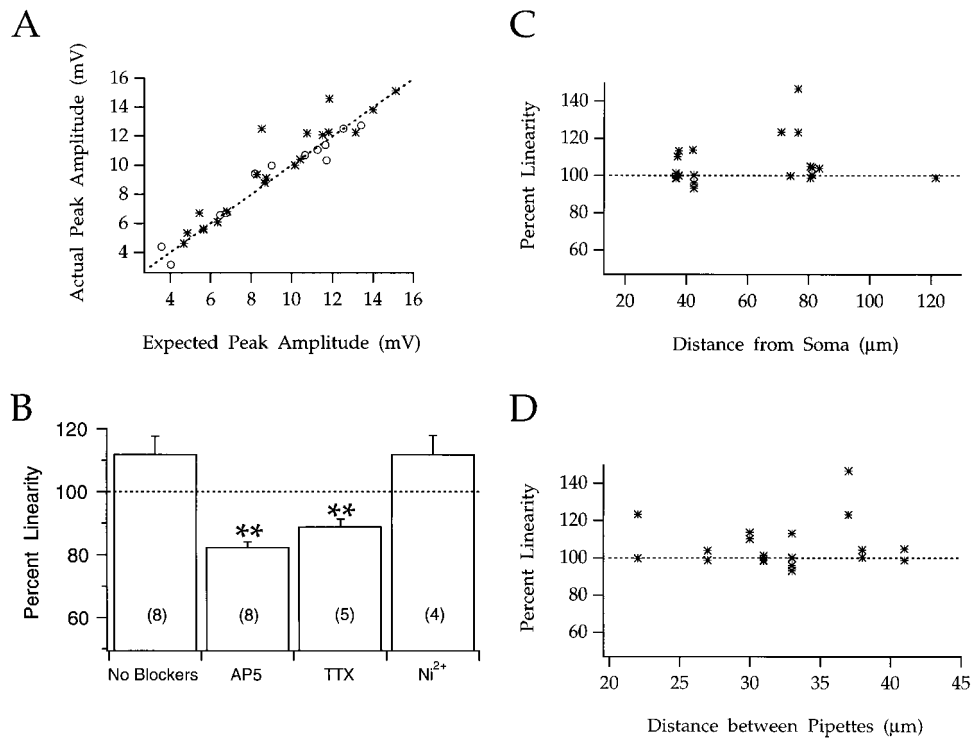


Figure 6. Mechanisms of Summation on Basal Dendrites

(A) Iontophoresis potentials (stars, 20 experiments; 12 cells) or EPSPs (circles, 12 experiments; 7 cells) on basal dendrites add linearly at both small and large amplitudes. Iontophoresis was done on the same basal dendrite. Dotted line shows expected linear summation.  
 (B) Role of NMDA, Na<sup>+</sup>, and Ca<sup>2+</sup> channels on summation of inputs in basal dendrites. Again, bath application of the NMDA receptor blocker AP-5 (100 μM) or the Na<sup>+</sup> channel blocker TTX (5 μM) blocked the linearity, whereas the Ca<sup>2+</sup> channel blocker Ni<sup>2+</sup> (1 mM) did not have a significant effect.  
 (C) Summation of large (10–15 mV expected) excitatory events on basal dendrites is not dependent on the distance of those inputs from the soma.  
 (D) Summation of iontophoretic stimuli on basal dendrites is also not affected by the distance separating the pipettes.

be due to a reduction in the driving force or a shunt of the iontophoretic potential.

#### Summation under Trains of Inputs or Action Potentials Is Also Linear

Are our results applicable to the physiological regimes that CA1 neurons experience *in vivo*? Our experiments used iontophoretic inputs or EPSPs of relatively small amplitudes (2–10 mV). Based on quantal analysis (Otmakhov et al., 1993) and on our own measurements of single spine currents (Yuste et al., 1999), we estimate that these amplitudes correspond to the activation of 10–50 dendritic spines. *In vivo*, CA1 neurons display oscillatory responses approaching firing frequencies of hundreds of hertz (Buzsáki et al., 1992), and it is conceivable that under those conditions, when dendrites are bombarded with hundreds or even thousands of inputs, input summation may differ from the cases we studied in brain slices.

We reasoned that heightened synaptic activation could produce three major differences in the functional properties of CA1 dendrites between brain slices and *in vivo* experiments: (1) a different state of desensitization of synaptic receptors, due to past synaptic activity, (2) a different activation or inactivation state of dendritic

channels, due to action potential invasion of the dendrites, and (3) changes in passive cable properties secondary to the activation of synaptic receptors or dendritic channels. To test the effect of previous synaptic activity on summation, we examined the integration in apical dendrites of trains of iontophoretically induced depolarizations or EPSPs (ten stimuli at 25 Hz; Figure 8A). With either method, while the first pulse was sub-linear, subsequent joint responses were increasingly linear (88% ± 3% for first pulse, 106% ± 6% for last pulse, n = 8). Linear summation after continuous excitation was also observed when applying a single iontophoretic input from one pipette coincident with the final event of a train from the second pipette (100% ± 3%, n = 3, 20–25 Hz, ten pulses). These results point out the importance of the temporal structure of the inputs for integration. Specifically, coincident activity after sustained excitation would be enhanced relative to isolated coincident events. Based on our previous data, we presume that the mechanism underlying this temporal effect is the inactivation kinetics of dendritic I<sub>A</sub> K<sup>+</sup> channels (Hoffman et al., 1997). To investigate further the temporal dependency of the apical dendrite sublinearity, we performed temporal shift experiments in which one input preceded the other. These experiments revealed that a small difference in the timing of the two inputs abolished the sublinearity (Figure 8B), indicating that the “window” in

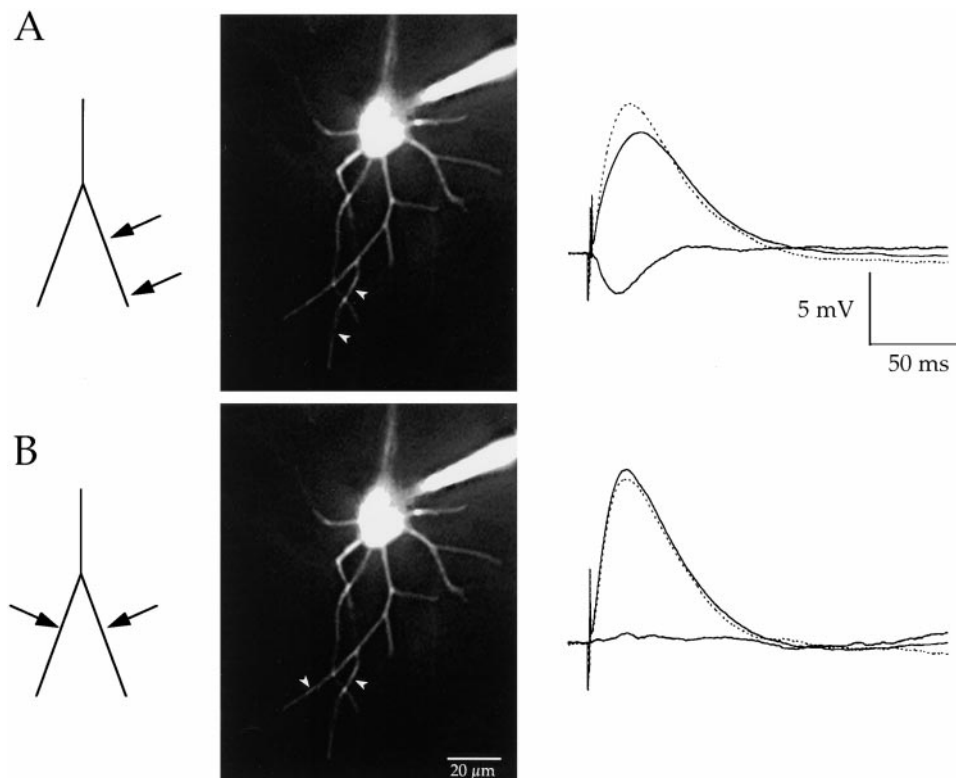


Figure 7. A Local Sublinearity Is Present in Basal Dendrites under AP-5

(A) Sublinear summation of two inputs in the same branch of a basal dendrite in AP-5 (100  $\mu$ M). Left panel, schematic representation of the experiment. Middle panel, photomicrograph taken during a representative experiment. The soma (top) and the recording patch pipette (top right) are clearly visible. The position of the iontophoresis pipettes on a secondary branch of a basal dendrite is indicated by white arrows. Right panel, electrophysiological results. Top solid line, combined effect; dotted line, expected effect, and bottom solid line, subtracted waveform showing the time course of the sublinearity. Note how the peak of the combined depolarization is shifted in time with respect to the expected depolarization.

(B) The sublinearity disappears if one pipette is moved to a neighboring branch. Left panel, representation of the experiment with two inputs on neighboring branches from a basal dendrite. Middle panel, the bottom iontophoresis pipette has now been moved to a neighboring branch to the left of the first one. Right panel, summation is practically linear.

which there are sublinear interactions is open for <40 ms. This interval is also consistent with the kinetics of a transient  $I_A K^+$  conductance activated by the iontophoresis potential.

To test if previous action potential history affected input summation, we generated long trains of action potentials (20 Hz, 5 s), produced by repeated injection of depolarizing current in the soma (100–200 pA, 2–5 ms) and then studied summation of EPSPs 20 ms after the last action potential (Figure 8C). To avoid stimulation of common inputs, we restricted our analysis to EPSPs generated by stimulation of the stratum radiatum and oriens, which would produce activation of apical/oblique and basal inputs, respectively. We found that summation of peak amplitudes was linear ( $98 \pm 3$ ,  $n = 3$ ; Figure 8C, inset) and not significantly different from summation of radiatum/oriens EPSPs elicited without trains of action potentials.

Finally, to explore the effect of the combination of intense synaptic stimulation and action potentials on summation, we generated trains of large iontophoretic inputs, which were suprathreshold for action potential activation for most events, and examined the summation

of subthreshold iontophoretic inputs interspersed in the trains. We found that summation of subthreshold inputs in the midst of intense synaptic activity is still linear (Figure 8D; three of three cells).

## Discussion

### Linear Summation of Inputs in CA1 Pyramidal Neurons

This study was motivated by the classic work of Rall, who in 1964 proposed that inputs on the same dendritic branch would reduce each other's effect, whereas inputs onto different branches would sum linearly. In this scenario, the role of the dendritic branching would be to ensure the independent processing of inputs. To our knowledge, this simple but important question had not been directly addressed experimentally, and therefore we decided to test whether this effect was present in the summation of two excitatory inputs on hippocampal pyramidal neurons using iontophoretic application of glutamate and EPSPs. Our main finding is that under different regimes of stimulation involving from a few inputs to probably hundreds of inputs, summation is

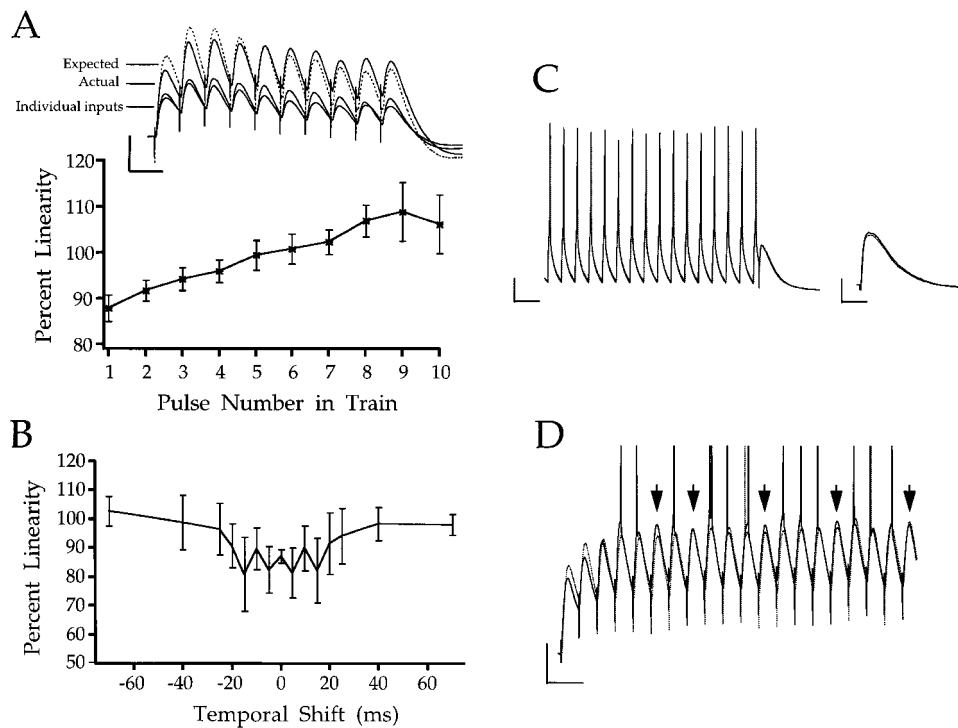


Figure 8. Linear Summation under Trains of Inputs or Action Potentials

(A) Linear summation after trains of inputs in the apical dendrite. Summation of iontophoretic or synaptic inputs (10 pulses, 25 Hz) was initially sublinear but became increasingly linear ( $n = 8$ ). Traces show an example of an individual event (lower solid lines) as well as the expected sum (dotted line) and actual combined train (top solid line). Scale bars, 5 mV, 50 ms.

(B) Temporal shifts reveal "window" for sublinearity. Timing between inputs was shifted from 0 to 70 ms, and the linearity of the combined amplitude at the peak of the later EPSP was measured (i.e., the combination of the tail of the first EPSP and the peak of the second EPSP). Sublinear integration was substantial only if inputs were within 40 ms of one another. Negative values indicate that the distal input preceded the more proximal input. Thirty-seven cells were used, with each point representing data from between 2 and 20 different cells, with most points  $n > 5$ .

(C) Linear summation of EPSPs after trains of action potentials. End of a train of somatically induced action potentials (20 Hz, 5 s), followed 20 ms later by two EPSPs in the oriens and radiatum, respectively. Scale bars, 10 mV, 50 ms. Inset shows summed EPSPs at expanded time and voltage scale, after the train of action potentials has been subtracted. Nearly perfect linear summation is evident. Scale bars, 5 mV, 25 ms.

(D) Linear summation during trains of inputs and action potentials. Train of iontophoretically induced depolarizations at two positions along the apical dendrite. As in (A), summation is initially sublinear but becomes linear, and this process is not changed by the presence of action potentials preceding the subthreshold of the depolarization (arrows). Scale bars, 5 mV, 100 ms.

overwhelmingly linear and that this linearity results from the balanced activation of different types of active conductances that boost or shunt the inputs. Under control conditions, the only significant nonlinearity observed was a small sublinear summation of two inputs in the distal apical dendrite that disappears with trains of inputs and appears mediated by  $I_A$  conductances. Thus, with the possible exception of the activation of basal dendrites in the presence of AP-5, in our experiments we have failed to detect the local shunt predicted from the reduction of either the driving force or input resistance of the dendrite. We are puzzled by this, since we estimate that two 10 mV EPSPs impinging simultaneously on a dendrite would produce a sublinear summation of ~40% (our own calculations and G. Major, personal communication).

Why isn't this local sublinearity present in our data? One possibility is that the effective space constant ( $\lambda$ ) for EPSPs or iontophoretic depolarization is smaller than

the spatial resolution of our technique ( $\sim 10 \mu\text{m}$  between inputs). For example, inputs that are very close, i.e., on the same spine or immediately adjacent on a dendritic shaft, may interact, but a small distance of separation could prevent attenuation. A  $\lambda$  of this magnitude, however, seems at least an order of magnitude smaller than those estimated in previous studies (Major et al., 1994; Stuart and Spruston, 1998). Although experiments using two photon microscopy to localize inputs on individual spines could be used to test whether EPSPs on neighboring spines interact (Yuste and Denk, 1995; Yuste et al., 1999), we judge it unlikely that a local sublinearity would have such a short range of action. A second possibility to explain the lack of local sublinearity in our data, and the one we favor, is that active conductances, by substantially modifying the EPSP, may compensate the effect of the local shunt or driving force reduction predicted by cable theory. In fact, in our previous experiments with culture neurons, after  $\text{Na}^+$ ,  $\text{K}^+$ , NMDA, and

Ca<sup>2+</sup> conductances were blocked, we revealed an underlying sublinearity that could be mediated by a reduction of the driving force (Cash and Yuste, 1998). Another indication that this may be the case are our results in the basal dendrites, where after blockade of NMDA receptors or Na<sup>+</sup> channels, we uncovered an underlying sublinearity that appears to be restricted to individual branches and produces a shift in the time-to-peak of the combined responses. We therefore propose that the cable sublinearity actually exists in pyramidal neurons but that it is normally compensated by the action of active conductances.

### Functional Compartmentalization of the Dendritic Tree

Do dendrites serve to isolate inputs? Although our results indicate that the precise dendritic morphology does not play a major role in neuronal integration of excitatory inputs, we still wondered if there was a general logic to the morphological effects on summation found throughout the dendritic tree. First, the presence of branch points did not seem to affect summation, since the oblique-apical results were indistinguishable from the apical-apical results. Second, the dichotomy between basal or apical dendritic tree could not fully explain the different effects we observed, because inputs on the proximal apical tree summated more linearly, like basal inputs. Nevertheless, we noticed that the input location along the basal-soma-apical dendritic axis correlated with the result of the summation, with progressively more sublinear summation in the distal apical dendritic tree. Based on these results, we hypothesize that pyramidal neurons have two functional compartments, one composed of the soma, basal dendrites, and the proximal apical dendrite, where input summation is essentially linear, and another consisting of the distal apical dendritic tree, with sublinear summation. Consistent with this idea, distal apical dendrites can sustain local electrogenesis (Wong et al., 1979; Amitai et al., 1993; Yuste et al., 1994; Schiller et al., 1997). Moreover, excitatory (Amaral and Witter, 1989) and inhibitory (Buhl et al., 1994) afferents follow a laminar pattern of segregation that respects these compartments. Thus, more than any other morphological parameter, the laminar position of synapses along the dendritic tree may determine their integration arithmetic.

Nevertheless, larger effects of dendritic morphology on input integration may occur under circumstances that we have not yet tested. For instance, input position may be important for summation of excitatory and inhibitory inputs (Koch et al., 1983). Indeed, the highly specific placement of inhibitory contacts in the dendritic tree (Buhl et al., 1994) suggests that their integrative interactions may be position-dependent, although this has not yet been explored experimentally. Another positional effect may arise during interaction of EPSPs with back-propagating action potentials that could invade only a subset of the dendritic tree (Spruston et al., 1995; Hoffman et al., 1997), although in our experiments with trains of action potentials, we have not noticed any significant deviation from linearity in summation of radiatum/oriens inputs. Finally, as in Purkinje cells (Llinas and Sugimori,

1980; Ross and Werman, 1987), dendritic electrogenesis may be restricted to specific dendrites or dendritic branches (R. Y., unpublished data). Further experiments are needed to address these possibilities.

### Active Conductances Shape Input Summation

Underlying the summation of excitatory potentials, we found evidence for the involvement of different sets of active conductances. Because these effects are revealed by the particular position of the inputs, we reason that these active conductances must be dendritic. Inputs on distal apical dendrites added sublinearly, and this sublinearity was blocked by 4-AP. Thus, it is likely to result from the activation of dendritic I<sub>A</sub> K<sup>+</sup> channels (Hoffman et al., 1997). In basal and oblique dendrites, however, there seems to be a substantial enhancement of the EPSPs produced by NMDA, Na<sup>+</sup>, and in the oblique dendrites, also Ca<sup>2+</sup> channels, which are all thought to exist throughout the dendritic tree of pyramidal neurons (Monaghan et al., 1983; Johnston et al., 1996). Indeed, it is surprising that supralinearity was not observed, since Na<sup>+</sup> and Ca<sup>2+</sup> channels boost subthreshold EPSPs (Liposky et al., 1996; Gillessen and Alzheimer, 1997; Hoffman et al., 1997). The discrepancies between our results and the predictions from these studies are difficult to resolve. There were no significant differences in experimental protocols such as age, temperature at which the experiments were performed, or intracellular and extracellular solutions. One possibility is that the boost provided by Na<sup>+</sup> and Ca<sup>2+</sup> channels for single inputs is essentially linear over the range of combined inputs tested. As a result, individual inputs are boosted slightly, but the combination of similarly sized synaptic inputs is not further amplified. On the other hand, it is also conceivable that, as discussed above, there are in fact local nonlinearities, but that they are counteracted downstream by other mechanisms, maintaining a nearly linear integration, as measured at the soma. Active dendritic conductances may, paradoxically, be employed to linearize inputs regardless of their position and thus counteract the effects of the neuron's passive cable structure and maybe even local electrogenic processes (Bernarder et al., 1994). This active mechanism of maintaining linearity can explain the paradoxically linear summation seen *in vivo*, even with large EPSPs (Jagadeesh et al., 1991, 1997).

Finally, one of the consequences of our findings is that the neuron must regulate the location and density of its dendritic conductances very accurately during its development. We find it astonishing that the delicate balance among different sets of intrinsically nonlinear elements is controlled in such a way that robust linear summation is ensured. Perhaps activity-dependent rules of development, applied to the conductances of individual neurons (Turrigiano et al., 1998), may contribute to the precision in the assembly of the dendritic tree.

### Computational Relevance of Linear Summation

These results imply that CA1 neurons are built to ensure an essentially linear integration for most inputs, as measured at the soma. What might be the function of linear summation? A linear arithmetic could provide neurons

with a system for keeping an exact count of active inputs and also enable independent processing of multiple channels of information by preventing input interaction. Indeed, studies in the visual system have resulted in linear models of visual processing (Wandell, 1995). For example, using intracellular recordings from simple cells in the cat visual cortex, Jagadeesh and colleagues (1991) tested a linear model of directional selectivity. Fluctuations in membrane potential evoked by moving stimuli were accurately predicted by the linear sum of responses to stationary stimuli supporting linear synaptic summation. In neural networks with distributed connectivity, linear summation of inputs could enable a group of neurons to function as an associative memory matrix (Hopfield, 1982; Hopfield and Tank, 1986). In the hippocampus, it is conceivable that the linear summation that we find at the cellular level, together with input-specific learning rules, could be used to associative different inputs within a sparse connectivity matrix (Brown and Zador, 1990; Skaggs and McNaughton, 1996). Therefore, the apparent complex dendritic architecture and active conductances present in dendrites from CA1 pyramidal neurons may actually make their function relatively simple and robust, perhaps a necessary feature for building reliable, stable neuronal networks.

#### Experimental Procedures

Hippocampal slices from postnatal day (P) 14–27 (unless otherwise noted) Sprague-Dawley rats were prepared by standard methods. Rats were either cryoanesthetized (P, <9 or anesthetized (P, >9) with Ketamine/Xylazine (120 mg/kg, 10 mg/kg, respectively). Animals older than P18 were intracardially perfused with cold “high sucrose” artificial cerebrospinal fluid (ACSF) containing (in mM): KCl, 2; CaCl<sub>2</sub>, 2; MgSO<sub>4</sub>, 2; NaHCO<sub>3</sub>, 26; NaH<sub>2</sub>PO<sub>4</sub>, 1.25; D-glucose, 10; and sucrose, 206. Animals were decapitated, and the brain was quickly removed and placed in ACSF. Coronal slices, 300 μm thick, were cut with a vibratome (TPI) and incubated at 35°C for 30 min in a submerged slice chamber with ACSF containing (in mM): NaCl, 126; KCl, 3; CaCl<sub>2</sub>, 2; MgSO<sub>4</sub>, 1; NaHCO<sub>3</sub>, 26; NaH<sub>2</sub>PO<sub>4</sub>, 1; and D-glucose, 10. Slices were then incubated at room temperature in ACSF for up to 12 hr. In some experiments, 100 μM TROLOX (±, Sigma) was added to the ACSF to prevent photo damage (Sheenen et al., 1996). Whole-cell recordings (Axopatch 1D, Dagan BVC-700) from CA1 pyramidal neurons were made with conventional patch pipettes (≈4–7 MΩ tip resistance) filled with (in mM): NaCl, 5; KCl, 10; HEPES, 10; KMeSO<sub>3</sub>, 140; Mg-ATP, 2.5; and EGTA, 1.1. To visualize the dendritic tree, rhodamine dextran (25–50 μM, 3000 MW, Molecular Probes) was included in the pipette solution. In all experiments, potentials were recorded at ≈28°C, and the membrane potential was maintained near –60 mV by current injection. Experiments testing summation of iontophoretic inputs on the apical dendrite were also performed at ≈34°C, but the results were not significantly different (n = 5). Membrane potential was filtered at 1 kHz, digitized, stored, and analyzed by Superscope and an A/D board (GW Instruments) and corrected for a 7 mV liquid junction potential. Time constants were measured by fitting a single exponential to the beginning of a hyperpolarizing pulse.

Iontophoresis pipettes were pulled to a fine tip with ≈150 MΩ resistance when filled with 2.5 M NaCl. Pipettes were filled with 250 mM sodium glutamate in Milli-Q water (pH 8.0). For experiments in hippocampal slices, iontophoresis pipettes were bent ≈90° with a microforge and filled with 250 mM NaGlutamate and 100 μM rhodamine dextran to visualize their tips. In some cases, the pipette tips were coated with a silane solution (Rain-X) to decrease the amount of cellular debris that would build up and block the tip. Four stimulus isolation units and a Master-8 controller (AMPI) were used to provide holding current (≈1–10 nA) and ejection current (≈100 nA) of various durations to the pipettes. Potentials of relatively large amplitude

and fast rise time were obtained with ejection currents between 0.04 and 5 ms. Action potentials or significantly larger and longer potentials could always be achieved by increasing the ejection current amplitude or duration, indicating that neither the neuron nor the amplifier was saturated by combined iontophoretic events. Simultaneous application of CNQX (20 μM) and AP-5 (100 μM) completely blocked the potentials elicited by glutamate iontophoresis. Extracellular stimulation electrodes were patch pipettes filled with ACSF. Voltage steps were supplied by stimulus isolation units controlled by the Master-8. To eliminate confounding effects of inhibitory postsynaptic potentials (IPSPs), synaptic stimulation experiments were carried out in the presence of 10 μM bicuculline, a GABA<sub>A</sub> antagonist. Each experiment consisted of multiple trials (between 5 and 15) at a given pipette position and/or amplitude of glutamate response. Throughout this paper, measurements are expressed as the mean ± SEM. For analysis, distances were measured from the soma along the dendrite. For inputs on the same branch or on an apical and a basal, the distance used is the mean distance of the two inputs. For inputs on different branches, the distance is measured from the common branch point.

#### Acknowledgments

We thank G. Major for his simulations and stimulating comments; M. Goodman, K. Holthoff, A. Majewska, and A. Tsiola for comments; and S. Firestein, D. Kelley, S. Siegelbaum, and G. Shepherd for valuable discussions. This work was supported by the Human Frontier Science Project program, the EJLB and Beckman Foundations, and by the National Eye Institute (EY 111787–01A1).

Received June 16, 1998; revised January 12, 1999.

#### References

- Amaral, D.G., and Witter, M.P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 37, 571–591.
- Amitai, Y., Friedman, A., Connors, B.W., and Gutnick, M.J. (1993). Regenerative activity in apical dendrites of pyramidal cells in neocortex. *Cereb. Cortex* 3, 26–38.
- Bernarder, O., Koch, C., and Douglas, R.J. (1994). Amplification and linearization of distal synaptic input to cortical pyramidal cells. *J. Neurophysiol.* 72, 2743–2753.
- Brown, T.H., and Zador, A.M. (1990). Hippocampus. In *The Synaptic Organization of the Brain*, G.M. Shepard, ed. (New York: Oxford University Press), pp. 346–388.
- Buhl, E.H., Halasy, K., and Somogyi, P. (1994). Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic sites. *Nature* 368, 823–828.
- Burke, R.E. (1967). Composite nature of the monosynaptic excitatory postsynaptic potential. *J. Neurophysiol.* 30, 1114–1137.
- Buzsáki, G., Horvath, Z., Urioste, R., Hetke, J., and Wise, K. (1992). High-frequency network oscillations in the hippocampus. *Science* 256, 1025–1027.
- Cash, S., and Yuste, R. (1998). Input summation by cultured pyramidal neurons is linear and position-independent. *J. Neurosci.* 18, 10–15.
- Gillessen, T., and Alzheimer, C. (1997). Amplification of EPSPs by low Ni<sup>2+</sup> and amiloride-sensitive Ca<sup>2+</sup> channels in apical dendrites of rat CA1 pyramidal neurons. *J. Neurophysiol.* 77, 1639–1643.
- Hoffman, D.A., Magee, J.C., Colbert, C.M., and Johnston, D. (1997). Potassium channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* 387, 869–875.
- Hopfield, J.J. (1982). Neural networks and physical systems with emergent collective computational abilities. *Proc. Natl. Acad. Sci. USA* 79, 2554–2558.
- Hopfield, J.J., and Tank, D.W. (1986). Computing with neural circuits: a model. *Science* 233, 625–633.
- Jack, J.J.B., Noble, D., and Tsien, R.W. (1975). *Electric Current Flow in Excitable Cells* (London: Oxford University Press).

- Jagadeesh, B., Wheat, H.S., and Ferster, D. (1991). Linearity of summation of synaptic potentials underlying direction selectivity in simple cells of the cat visual cortex. *Science* 262, 1901–1904.
- Jagadeesh, B., Wheat, H.S., Kontsevich, L.L., Tyler, C.W., and Ferster, D. (1997). Direction selectivity of synaptic potentials in simple cells of the cat visual cortex. *J. Neurophysiol.* 78, 2772–2789.
- Johnston, D., Magee, J.C., Colbert, C.M., and Christie, B.R. (1996). Active properties of neuronal dendrites. *Annu. Rev. Neurosci.* 19, 165–186.
- Koch, C., Poggio, T., and Torre, V. (1983). Nonlinear interactions in a dendritic tree: localization, timing and role in information processing. *Proc. Natl. Acad. Sci. USA* 80, 2799–2802.
- Kuno, M., and Miyahara, J.T. (1969). Non-linear summation of unit synaptic potentials in spinal motoneurons of the cat. *J. Physiol.* 201, 465–477.
- Langmoen, I.A., and Andersen, P. (1983). Summation of excitatory postsynaptic potentials in hippocampal pyramidal neurons. *J. Neurophysiol.* 50, 1320–1329.
- Larkman, A.U. (1991). Dendritic morphology of pyramidal neurons of the visual cortex of the rat: I. branching patterns. *J. Comp. Neurol.* 306, 307–319.
- Liposky, R., Gillessen, T., and Alzheimer, C. (1996). Dendritic Na<sup>+</sup> channels amplify EPSPs in hippocampal CA1 pyramidal cells. *J. Neurophysiol.* 76, 2181–2191.
- Llinás, R.R. (1988). The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. *Science* 242, 1654–1664.
- Llinás, R., and Sugimori, M. (1980). Electrophysiological properties of in vitro Purkinje cell dendrites in mammalian cerebellar slices. *J. Physiol.* 305, 197–213.
- Major, G., Larkman, A.U., Jonas, P., Sakmann, B., and Jack J.J. (1994). Detailed passive cable models of whole-cell recorded CA3 pyramidal neurons in rat hippocampal slices. *J. Neurosci.* 14, 4613–4638.
- Malenka, R.C., and Nicoll, R.A. (1997). Silent synapses speak up. *Neuron* 19, 473–476.
- Monaghan, D.T., Holets, V.R., Toy, D.W., and Cotman, C.W. (1983). Anatomical distributions of four pharmacologically distinct 3H-L-glutamate binding sites. *Nature* 306, 176–179.
- Otmakhov, N., Shirke, A.M., and Malinow, R. (1993). Measuring the impact of probabilistic transmission on neuronal output. *Neuron* 10, 1101–1111.
- Rall, W. (1964). Theoretical significance of dendritic trees for neuronal input-output relations. In *Neural Theory and Modeling*, R.F. Reiss, ed. (Stanford: Stanford University Press), pp. 73–97.
- Rall, W. (1995). *The theoretical foundation of dendritic function* (Cambridge, Massachusetts: MIT Press).
- Ramón y Cajal, S. (1904). *La Textura del Sistema Nervioso del Hombre y los Vertebrados* (Madrid: Moya).
- Ross, W.N., and Werman R. (1987). Mapping calcium transients in the dendrites of purkinje cells from guinea-pig cerebellum in vitro. *J. Physiol.* 389, 319–336.
- Schiller, J., Schiller, Y., Stuart, G., and Sakmann, B. (1997). Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *J. Physiol. (Lond)* 505, 605–616.
- Sheenen, W.J.J.M., Makings, L.R., Gross, L.R., Pozzan, T., and Tsien, R.Y. (1996). Photodegradation of indo-1 and its effect on apparent Ca concentrations. *Chem. Biol.* 3, 765–774.
- Skaggs, W.L., and McNaughton, B.L. (1996). Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science* 271, 1870–1873.
- Skydsgaard, M., and Hounsgaard, J. (1994). Spatial integration of local transmitter responses in motoneurons of the turtle spinal cord in vitro. *J. Physiol.* 479, 233–246.
- Spruston, N., Schiller, Y., Stuart, G., and Sakmann, B. (1995). Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. *Science* 286, 297–300.
- Stuart, G., and Spruston, N. (1998). Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *J. Neurosci.* 18, 3501–3510.
- Thomson, A.M., Girdlestones, D., and West, D.C. (1988). Voltage-dependent currents prolong single-axon postsynaptic potentials in layer 3 pyramidal neurons in rat neocortical slices. *J. Neurophysiol.* 60, 1896–1907.
- Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C., and Nelson, S.B. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391, 892–896.
- Wandell, B.A. (1995). *Foundations of Vision* (Sunderland, Massachusetts: Sinauer).
- Wong, R.K.S., Prince, D.A., and Basbaum, A.I. (1979). Intradendritic recordings from hippocampal neurons. *Proc. Natl. Acad. Sci. USA* 76, 986–990.
- Wu, G.-Y., Malinow, R., and Cline, H.T. (1996). Maturation of a central glutamatergic synapse. *Science* 274, 972–976.
- Yuste, R., and Denk, W. (1995). Dendritic spines as basic units of synaptic integration. *Nature* 375, 682–684.
- Yuste, R., and Tank, D.W. (1996). Dendritic integration in mammalian neurons, a century after Cajal. *Neuron* 16, 701–716.
- Yuste, R., Gutnick, M.J., Saar, D., Delaney, K.D., and Tank, D.W. (1994). Calcium accumulations in dendrites from neocortical neurons: an apical band and evidence for functional compartments. *Neuron* 13, 23–43.
- Yuste, R., Majewska, A., Cash, S., and Denk, W. (1999). Mechanisms of calcium influx into spines: heterogeneity among spines, coincidence detection by NMDA receptors and optical quantal analysis. *J. Neurosci.* 19, 1–12.