ed and used at 1:4 dilution in the IFN-γ assay (*17*) in the presence or absence of anti-IGIF (25 μg/ml) (*6*).
21. Wild-type or ICE-deficient mice were primed with *P. acnes* (*20*). Seven days later, mice were exposed to LPS (1 μg, intravenously). In some experiments, recombinant mature IGIF (1 μg) or protein G–purified anti-IGIF (250 μg) was coinjected with LPS; sera were collected 3 hours after LPS exposure.
22. Reduced IFN-γ was also observed in *Listeria*-infected (N. M. Tsuji *et al.*, in preparation) and LPS-exposed (G. Ku *et al.*, in preparation) ICE$^{-/-}$ mice.
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A Synaptically Controlled, Associative Signal for Hebbian Plasticity in Hippocampal Neurons

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The role of back-propagating dendritic action potentials in the induction of long-term potentiation (LTP) was investigated in CA1 neurons by means of dendritic patch recordings and simultaneous calcium imaging. Pairing of subthreshold excitatory postsynaptic potentials (EPSPs) with back-propagating action potentials resulted in an amplification of dendritic action potentials and evoked calcium influx near the site of synaptic input. This pairing also induced a robust LTP, which was reduced when EPSPs were paired with non–back-propagating action potentials or when stimuli were unpaired. Action potentials thus provide a synaptically controlled, associative signal to the dendrites for Hebbian modifications of synaptic strength.

Recent evidence for the presence of voltage-gated Na^+, Ca^{2+}, and K^+ channels in dendrites and the active propagation of action potentials from the axon into the dendrites has required a reevaluation of the mechanisms of synaptic integration and synaptic plasticity in central neurons (*1*). In hippocampal neurons, LTP is thought to occur in response to the simultaneous activation of both pre- and postsynaptic elements (*2*, *3*). Most LTP induction protocols, however, involve prolonged depolarizations of the postsynaptic neuron (*4*). Thus, it is not clear whether under more physiological conditions postsynaptic action potentials are important for LTP induction, as originally suggested by Hebb (*5*). In Hebbian learning theories, correlated synaptic input and action potential output are associated with increases in synaptic strength (*6*). The relatively large physical distance separating the input (dendrites) from the output (axon) creates the need for a rapid feedback signal capable of forming an association between the synaptic input and the action potential output of the neuron. The back-propagating dendritic action potential appears to be ideally suited for such an associative signal. Axonally initiated action potentials (*7*) propagate rapidly into the soma and dendrites, providing large membrane depolarizations and substantial increases in dendritic intracellular calcium ion concentration ($[Ca^{2+}]_i$) (*8*, *9*).

Back-propagating action potentials decline in amplitude with distance from the cell body (*8*, *10*) and fail to propagate beyond certain distal branch points during repetitive firing (*8*). We found that pairing of axonally initiated action potentials with subthreshold EPSPs increased dendritic action potential amplitude and Ca^{2+} influx (Fig. 1) (*11*, *12*). A subthreshold EPSP train produced a small and highly localized increase in $[Ca^{2+}]_i$ (2% $\Delta F/F$ in the region labeled with an asterisk), whereas the unpaired action potential train induced a more widespread, but still relatively small, increase in $[Ca^{2+}]_i$ (5% $\Delta F/F$) (Fig. 1A). Pairing of synaptic stimulation and back-propagating action potentials, however, resulted in an increase in $[Ca^{2+}]_i$ that was significantly larger than the simple sum of the two independent Ca^{2+} signals (10% $\Delta F/F$) (Fig. 1A). The amount of the pairing-induced increase in action potential amplitude and Ca^{2+} influx increased progressively with distance from the cell body (Fig. 2, C and D). When EPSPs and action potentials occurred simultaneously, no significant changes in signal amplitudes were observed in somatic and proximal dendritic regions, whereas large, supralinear increases

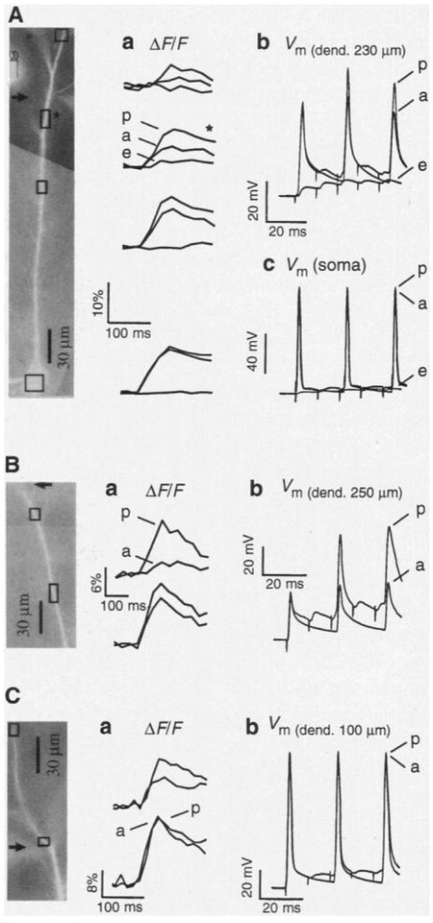

Fig. 1. Dendritic action potential amplitude and evoked Ca^{2+} influx are enhanced by simultaneous synaptic input. (**A**) (Aa) Optical recordings showing average $\Delta F/F$ from regions of the neuron delimited by the boxes shown at left. Traces are from progressively more proximal regions moving down the column in (b). Traces labeled e were recorded during subthreshold EPSPs; a, during unpaired action potentials; and p, during paired action potentials and EPSPs. Synaptic stimulation induced a significant increase in $[Ca^{2+}]_i$ in only the middle set of traces (*).The supralinear increase in $[Ca^{2+}]_i$ during paired EPSPs and action potentials is apparent in the more distal regions of the neuron. There was no such increase in the soma. (Ab) Electrical recordings from the dendrite showing supralinear summation of dendritic action potentials and EPSPs during paired stimulation. Traces are labeled as in (a). (Ac) Electrical recordings from the soma showing paired synaptic activity and action potential generation do not result in an increased action potential amplitude. Traces are labeled as in (b). (**B**) Dual electrical recordings from a neuron showing extreme supralinear summation in both the optical (Ba) and electrical (Bb) dendritic recordings. Unpaired dendritic action potentials appear to be nonregenerative. (**C**) Dual electrical recordings from a more proximal dendritic region. Pairing had little effect on Ca^{2+} entry in lower box (Ca) or on action potential amplitude (Cb). In the more distal optical recording (upper box, 200 μm), a larger increase in $[Ca^{2+}]_i$ during paired stimuli was observed. The locations of dendritic recording pipettes are labeled by arrows.

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were recorded from more distal regions (Fig. 1). This action potential and Ca^{2+} signal amplification were particularly prominent in areas where it appeared that back-propagating action potentials had become nonregenerative (Fig. 1B). Here dendritic action potential amplitudes had attenuated to such an extent that they were nearly too small to gate dendritic Ca^{2+} channels. In these regions, pairing EPSPs with action potentials increased action potential amplitudes by approximately twofold, whereas the associated increase in $[Ca^{2+}]_i$ was three- to fourfold (Fig. 2, C and D) (*13*).

The amplification of dendritic action potentials by subthreshold synaptic potentials could be mimicked by simple inward current injections into the dendrite. Current injections that depolarized the dendritic membrane increased action potential amplitudes and Ca^{2+} influx in a manner similar to that seen with paired action potentials and EPSPs (Fig. 2A). Furthermore, hyperpolarizing current injections had the opposite effect and reduced action potential amplitudes and Ca^{2+} influx (Fig. 2B) (*14*). The attenuation of action potential amplitude during back-propagation into the dendrites and the failure of propagation at some branch points (*8*) provide a highly nonuniform distribution of increases in $[Ca^{2+}]_i$ across the dendritic tree (*9*, *15*). The occurrence of EPSPs during the back-propagation can thus modulate and sculpt the potential and Ca^{2+} influx into dendritic branches that receive the synaptic input.

With the aid of simultaneous synaptic depolarization, back-propagating action potentials could provide the synaptic input region of a pyramidal neuron with a feedback signal that an output has occurred. Such a feedback signal is ideally suited for Hebbian modifications of synaptic strength (*16*). To test this idea, we examined the ability of subthreshold synaptic stimulation to induce changes in the efficacy of synaptic input in the absence of action potential generation. Short, theta-like trains (*17*) of subthreshold synaptic stimulation induced a small and localized increase in $[Ca^{2+}]_i$ into the apical dendrite but did not produce any persistent change in the EPSPs (Fig. 3, B and E). Trains of back-propagating action potentials alone, although inducing a larger and more widespread increase in $[Ca^{2+}]_i$, also did not result in any long-term increase in EPSP amplitudes (Fig. 3, C and E). The coincidence of both subthreshold synaptic stimulation and action potential generation, however, resulted in the largest and most widespread increase in dendritic $[Ca^{2+}]_i$ and induced significant LTP of the EPSPs (Fig. 3, D and E) (*18*).

The LTP was inhibited by Ca^{2+} channel antagonists nimodipine and Ni^{2+} (Fig. 3F) without any effect on baseline EPSPs. *N*-methyl-D-aspartate (NMDA) receptors also appeared to participate in the LTP. Although D,L-2-amino-5-phosphonovaleric acid (APV) at concentrations of up to 100 μM failed to block LTP completely, the addition of 20 μM MK-801 and preconditioning stimulation (1-Hz pairing for 10 to 20 s) to ensure the open-channel block of NMDA receptors (*19*) did prevent the induction of LTP (Fig. 3F). The LTP observed may thus have properties similar to both NMDA and non-NMDA–dependent LTP (*20*).

To examine the nature of the associative signal for LTP further, we blocked the back-propagation of somatic action potentials by transient application of tetrodotoxin (TTX) to a localized region of the proximal apical dendrite. A 500-ms application of 10 μM TTX to a small region of the apical dendrite just before action potential initiation caused back-propagating action potentials to fail distal to the blocked region (*21*). Relatively normal increases in $[Ca^{2+}]_i$ were recorded in neuronal regions proximal to the TTX application (Fig. 4B). This local blockade of dendritic Na^+ channels and action potential back-propagation was rapidly reversible, with normal propagation returning within 30 s of the initial TTX application, and had no effect on baseline EPSPs (*22*). Pairing of EPSP trains with non–back-propagating action potentials was ineffective for inducing LTP. Subsequent pairing without TTX application, however, resulted in a LTP of EPSP amplitudes (Fig. 4, D and E) (*23*). When the back-propagation of action potentials was inhibited by means of dendritic hyperpolarization (as in Fig. 2B), the amplification of action potentials by paired EPSPs was reduced, the ability of the action potentials to invade the synaptically active region of the dendrite was inhibited, and the incidence of pairing-induced LTP was decreased (Fig.

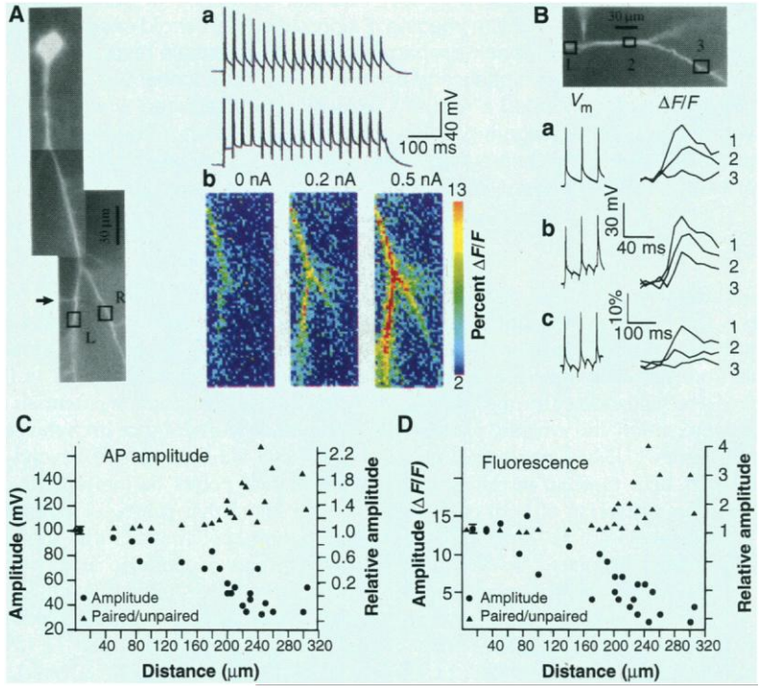

Fig. 2. Dendritic depolarization alone is sufficient to enhance action potential back-propagation. (**A**) (Aa) (Upper trace) Electrical recording from the dendrite (200 μm from the cell body; arrow) showing progressive decrease in action potential amplitude during a 40-Hz train. (Lower trace) Another action potential train during which 0.5 nA of inward current was injected, demonstrating that depolarizing current injection enhances action potential propagation. (Ab) Difference image of peak $\Delta F/F$ minus resting values showing that progressively large current injections increased the rise in $[Ca^{2+}]_i$ into both branches of the dendrite. (**B**) (Ba) Voltage and Ca^{2+} signals from the dendrite in response to unpaired action potential generation. There is no increase in $[Ca^{2+}]_i$ in the dendrite distal to the major branch point located 260 μm from the soma. (Bb) Pairing of EPSPs and action potentials increases action potential amplitude and rise in $[Ca^{2+}]_i$, particularly in dendritic regions distal to the major branch point. (Bc) Simultaneous hyperpolarizing current injection inhibits the amplifying effect of EPSP and action potential pairing. (**C**) Plot of action potential amplitude as a function of distance from the cell body (●). The amplifying effect of paired stimulation is expressed as paired action potential amplitude divided by unpaired action potential amplitude (▲) and is also plotted as a function of distance from the cell body. (**D**) Plot of action potential–induced increase in $[Ca^{2+}]_i$ as a function of distance from the cell body (●). The amplifying effect of paired stimulation on changes in $[Ca^{2+}]_i$ is expressed as paired $\Delta F/F$ divided by unpaired $\Delta F/F$ (▲) and is also plotted as a function of distance from the cell body.

4F) (*23*). This result demonstrates the importance of the action potential amplification by synaptic depolarization, without which the amplitude of back-propagating action potentials becomes insufficient to evoke both a large influx of Ca^{2+} and LTP in the more distal dendritic regions.

Using two different techniques to inhibit dendritic action potentials, we observed that synaptic activation that was not coincident with back-propagating action potentials did not result in LTP. In these instances, neuronal

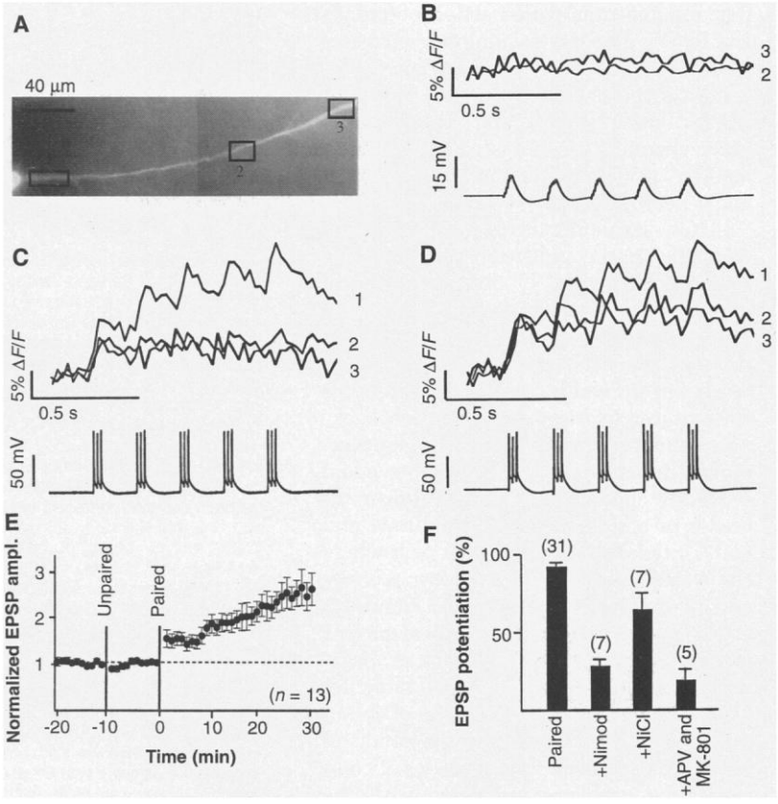

Fig. 3. Pairing of subthreshold synaptic stimulation and action potential trains induces LTP. (**A**) Fura-filled CA1 pyramidal neuron with somatic electrode. (**B**) (Upper traces) Optical recordings showing average $\Delta F/F$ from regions of the neuron delimited by the numbered boxes in (A) (box 1 omitted for clarity). Synaptic stimulation induced only a slight increase in $[Ca^{2+}]_i$ in the most distal region. (Lower trace) Somatic voltage showing a theta-like train of subthreshold EPSPs. (**C**) (Upper traces) Increase in $[Ca^{2+}]_i$ induced by a theta-like train of action potentials evoked by somatic current injection (2 nA, 2 ms). The increase in $[Ca^{2+}]_i$ is larger and more widespread than during subthreshold synaptic stimulation. (Lower trace) Somatic voltage showing the theta-like train of action potentials. (**D**) (Upper traces) Increase in $[Ca^{2+}]_i$ induced by pairing the trains of subthreshold EPSPs and action potentials. The pairing protocol resulted in an even larger increase in dendritic $[Ca^{2+}]_i$. (Lower trace) Somatic voltage showing the pairing of both action potential and subthreshold EPSP trains. (**E**) Grouped data showing normalized EPSP amplitude after unpaired and paired stimulation. (**F**) Induction of LTP by pairing of EPSPs and back-propagating action potentials is blocked by 50 μM APV + 20 μM MK-801, 10 μM nimodipine, or 50 μM Ni^{2+}. The amount of EPSP potentiation, plotted as percent of control, is shown for all cells under each condition. We calculated potentiation by dividing the average EPSP amplitude at 15 min after pairing by the average control EPSP amplitude. The percentage of cells showing >50% increase in EPSP amplitude 15 min after pairing under each condition: 17% (nimodipine), 42% (Ni^{2+}), and 20% (APV + MK-801).

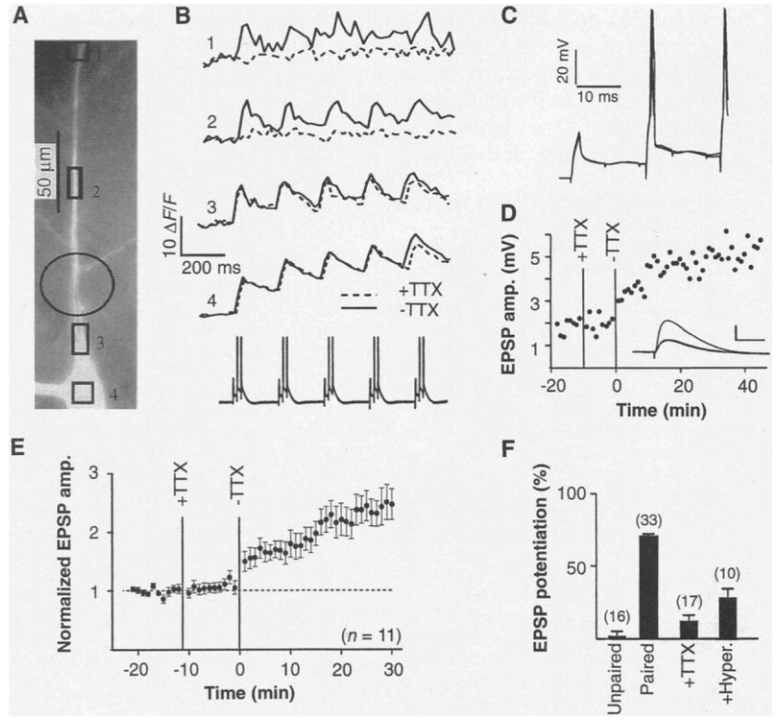

Fig. 4. Dendritic action potentials are required for induction of synaptic plasticity. (**A**) Fura-filled CA1 pyramidal neuron with somatic electrode. The approximate area of TTX application is shown by the oval. (**B**) Superimposed optical recordings from regions of the neuron delimited by the boxes in (A). Traces are from progressively more proximal regions moving down the column in (B). Dashed lines are the average $\Delta F/F$ during the pairing protocol given along with a transient application of 10 μM TTX to the dendrite. Solid lines are the average $\Delta F/F$ during the pairing protocol given without TTX application (~11 min later). The increase in $[Ca^{2+}]_i$ is similar in regions of the neuron proximal to the TTX application and is significantly reduced in those regions distal to TTX application site. (Lower trace) Somatic voltage during paired train. (**C**) Expanded somatic voltage recordings during the first burst of paired stimuli for trains with the TTX application and without. No appreciable differences are observable. The first current injection was subthreshold in all traces so that only two action potentials were evoked during each individual burst. (**D**) Plot of EPSP amplitude for the same neuron showing that paired stimuli without back-propagating action potentials do not modify EPSP amplitude, whereas subsequent paired stimuli with back-propagating action potentials result in a long-term, large increase in EPSP amplitude. (**Inset**) Average EPSPs for the last 2 min of each period (control, +TTX, −TTX). (**E**) Grouped data showing normalized EPSP amplitude after paired stimulation with and without TTX application. (**F**) Summary of mean LTP amplitude under various experimental conditions. The amount of EPSP potentiation, plotted as percent of control, is shown for all cells under each condition. We calculated potentiation by dividing the average EPSP amplitude at 10 to 15 min after stimulation by the average control EPSP amplitude.

output (action potentials) was coincident with synaptic input, but the associative link between the two was broken by prevention of action potential propagation into the dendrites. The feedback mechanism could have been the supralinear increase in $[Ca^{2+}]_i$ that resulted from paired action potentials and EPSPs. The largest nonlinear increases in $[Ca^{2+}]_i$ would presumably occur in the dendritic spines that had active synapses, where both NMDA and voltage-gated Ca^{2+} channels can be gated, as has been reported in dendritic spines of CA1 pyramidal neurons (*24*).

Thus, active dendrites play an important role in the induction of associative synaptic plasticity. Under physiological conditions, large synaptic inputs appear to form associations with weaker inputs not through their synaptic depolarization directly but instead by the action potentials they trigger in the axon that then back-propagate into the dendrites (*25*). Furthermore, the nature of the associative signal is readily apparent in the supralinear increase in action potential amplitude and Ca^{2+} influx into the dendrites near where the weak input occurs. Appropriately occurring synaptic input to the dendrites could function much like our local current injections and control the back-propagation of action potentials to distal branches (*14*). The retrograde propagation of action potentials to specific sites in the dendrites may help to resolve certain classes of computational problems associated with the timing of information flow within dendrites by the use of Hebbian modification rules (*16*, *26*). In addition, in the light of these results, these rules may need to include additional constraints so that inputs are modified only if they occur in the spatial domain of the dendrites to which action potentials are channeled.

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11. Hippocampal slices (400 μm) were prepared from 5- to 10-week-old Sprague-Dawley rats as described (*27*, *28*). Area CA3 was removed from each slice before recording. A Zeiss Axioskop, fitted with a 40× (Zeiss) or 60× (Olympus) water-immersion objective and differential interference contrast (DIC) optics, was used to view slices. Whole-cell patch-clamp recordings were made from visually identified CA1 pyramidal somata and dendrites with an Axoclamp 2A (Axon Instruments) and Dagan IX2-700 amplifier in "bridge" modes. Neurons exhibited a resting membrane potential (V_m) between −60 and −74 mV. The external recording solution contained 124 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM dextrose, and 0.01 mM bicculline, bubbled with 95% O_2–5% CO_2 at 35°C. Whole-cell recording pipettes (somatic: 2 to 4 megohm; dendritic: 7 to 10 megohm) were pulled from borosilicate glass and filled with 120 mM K gluconate, 20 mM KCl, 10 mM Hepes, 4 mM NaCl, 4 mM Mg adenosine 5′-triphosphate, 0.3 mM Mg guanosine 5′-triphosphate, and 14 mM phosphocreatine (pH 7.25 with KOH). Series resistance for somatic recordings was 8 to 20 megohm, whereas that for dendritic recordings was 15 to 50 megohm. Dendritic pipettes were coated with Sylgard. Axonally initiated action potentials were elicited with somatic depolarizing current injection, usually 2 nA for 2 ms.
12. To measure changes in $[Ca^{2+}]_i$, we included the fluorescent indicator fura-2 (80 to 100 μM) in the pipette solution and allowed each neuron to dialyze for ~10 min before optical recordings began. Using a cooled charge-coupled device (CCD) camera (Photometrics, Tucson, AZ) in a sequential frame transfer mode (*29*), we recorded high-speed fluorescence images from a 220- or 352-μm length of the neuron. Relative changes in $[Ca^{2+}]_i$ were quantified as changes in $\Delta F/F$, where F is fluorescence intensity before stimulation (after subtracting autofluorescence) and ΔF is the change from this value during neuronal activity (corrected for bleaching during the run). We determined the bleaching correction by measuring fluorescence of the neuron under non-stimulated conditions. We determined the tissue autofluorescence by an equivalent measurement at a parallel location in the slice that was away from the dye-filled neuron (*28*). We used 380-nm light (13-nm bandpass filter; Omega Optical) to excite fura-2. Sequential frame rate for optical recordings was one frame every 25 ms and pixels were binned in a 5 by 5 array.
13. Presumably the amplification of the action potentials in the dendrites was due to a combination of biophysical factors such as additional charging of the distributed dendritic capacitance and greater Na^+ channel activation. Similarly, the supralinear increase in $[Ca^{2+}]_i$ was likely to have resulted from the larger action potential amplitude and the steep voltage dependency for Ca^{2+} channel activation. In the soma, little or no amplification of the action potential could occur because the peak was already close to the Na^+ equilibrium potential. The amplitudes of the third action potentials in the train were compared in Fig. 2C.
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17. A train of subthreshold synaptic stimulation consisted of five bursts of synaptic stimuli given at 5 Hz. Each burst was composed of five stimuli at 100 Hz. The train was repeated twice with a 15-s interval. Likewise, five bursts of somatic current injections were given twice at 5 Hz. Each burst was composed of one to three current injections (usually 2 nA for 2 ms) at 20 Hz. Unpaired stimuli were EPSP and action potential trains given 1 min apart. Simultaneous presentation of these trains was given during paired stimuli. EPSP amplitudes were monitored for 10 to15 min after each unpaired, paired plus TTX, or hyperpolarization protocol. If after this period the EPSP amplitude was within 50% of control, a standard pairing protocol was given to the same neuron. After the pairing protocol, EPSP amplitudes were monitored for another 30 to 60 min.
18. Thirteen of 16 neurons (81%) showed >50% increase in EPSP amplitude 15 min after paired stimulation. Three neurons did not show this potentiation, and recordings were terminated after 15 min. These three neurons were not included in the plot shown in Fig. 3F. None of 16 neurons showed >50% increase in EPSP amplitude 15 min after unpaired stimulation (Fig. 4F).
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21. In control experiments in which dendritic recordings and Ca^{2+} imaging were performed distal to the TTX block, we found that the failure of action potential propagation distal to the TTX block and the decrease in Ca^{2+} signals in these regions were highly correlated.
22. TTX application did not reach the axons activated during the synaptic stimulation because there were no differences in EPSP plateau amplitudes during the stimulation with (at sites >250 μm from soma) (12.3 ± 0.9 mV, n = 11) or without (13.6 ± 1.0 mV, n = 11) TTX application. Somatic action potential amplitude (89.5 ± 2.7 mV versus 91.5 ± 2.5 mV, n = 11) and peak increases in $[Ca^{2+}]_i$ (15.8 ± 2.0% $\Delta F/F$ versus 16.3 ± 1.8% $\Delta F/F$, n = 11) were likewise unaffected by TTX application.
23. Only 2 of 17 (12%) neurons showed >50% increase in EPSP amplitude 15 min after paired stimulation with TTX application. No increases in distal dendrite $[Ca^{2+}]_i$ were observed in these two cells and they were not included in the group plotted in Fig. 4E. In the plus-TTX group, 11 of the 15 (73%) remaining neurons subsequently showed >50% increase in EPSP amplitude 15 min after a pairing protocol was given without TTX application. The four neurons not showing this potentiation were also not included in the plot shown in Fig. 4E. In the plus-hyperpolarization group, 2 of 10 (20%) neurons showed >50% increase in EPSP amplitude 15 min after paired stimulation with dendritic hyperpolarization. Very small increases in distal dendrite $[Ca^{2+}]_i$ were recorded in these two cells. Seven of the eight (88%) remaining neurons subsequently showed a >50% increase in EPSP amplitude 15 min after paired stimulation given without simultaneous dendritic hyperpolarization. See Fig. 4F for summary.
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Regulation of Synaptic Efficacy by Coincidence of Postsynaptic APs and EPSPs

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Activity-driven modifications in synaptic connections between neurons in the neocortex may occur during development and learning. In dual whole-cell voltage recordings from pyramidal neurons, the coincidence of postsynaptic action potentials (APs) and unitary excitatory postsynaptic potentials (EPSPs) was found to induce changes in EPSPs. Their average amplitudes were differentially up- or down-regulated, depending on the precise timing of postsynaptic APs relative to EPSPs. These observations suggest that APs propagating back into dendrites serve to modify single active synaptic connections, depending on the pattern of electrical activity in the pre- and postsynaptic neurons.

Repetitive activation of neuronal circuits can induce long-term changes in subsequent responses generated by synapses in many regions of the brain, and such plasticity of synaptic connections is regarded as a cellular basis for developmental and learning-related changes in the central nervous system (*1*, *2*). The actual triggers for synaptic modifications between two neurons are, however, unclear (*3*). Postsynaptic APs are initiated in the axon and then propagate back into the dendritic arbor of neocortical pyramidal neurons (*4*), evoking an activity-dependent dendritic Ca^{2+} influx (*5*) that could be a signal to induce modifications at the dendritic synapses that were active around the time of AP initiation. To test this hypothesis, we made dual whole-cell voltage recordings from neighboring, thick, tufted pyramidal neurons in layer 5 of the neocortex (Fig. 1A) for which the dendritic locations of synaptic contacts were known (*6*, *7*), and we investigated whether the postsynaptic AP could induce changes in unitary EPSP amplitudes.

When depolarizing current was injected only into the cell body of a presynaptic neuron to evoke a burst of APs, the resulting high-frequency train of subthreshold unitary EPSPs (Fig. 1B) failed to trigger changes in the average EPSP amplitudes (Fig. 1D), possibly because EPSP amplitudes decreased rapidly and a sufficiently large postsynaptic depolarization was not reached (*3*). When the postsynaptic neuron was further depolarized by current injection to produce a burst of APs during the EPSPs, then a persistent increase (>20%) was observed in 8 of 11 connections (Fig. 1, C and D; 94 ± 23% increase) (*8*, *9*).

To establish whether the occurrence of postsynaptic APs during EPSPs was indeed critical for the induction of the increase in EPSP amplitude, a number of control experiments were performed. Pairing of individual postsynaptic APs with EPSPs and without a sustained postsynaptic depolarization (Fig. 2A) induced a persistent increase in EPSP amplitudes (38 ± 9%; $n = 21$; 20 Hz; Fig. 2B) that was not associated with measurable changes in input resistance, current–AP discharge relation, or AP threshold. Neither bursts of postsynaptic APs alone nor high-frequency bursts of presynaptic APs induced persistent changes in EPSP amplitudes (Fig. 2B). The increase in

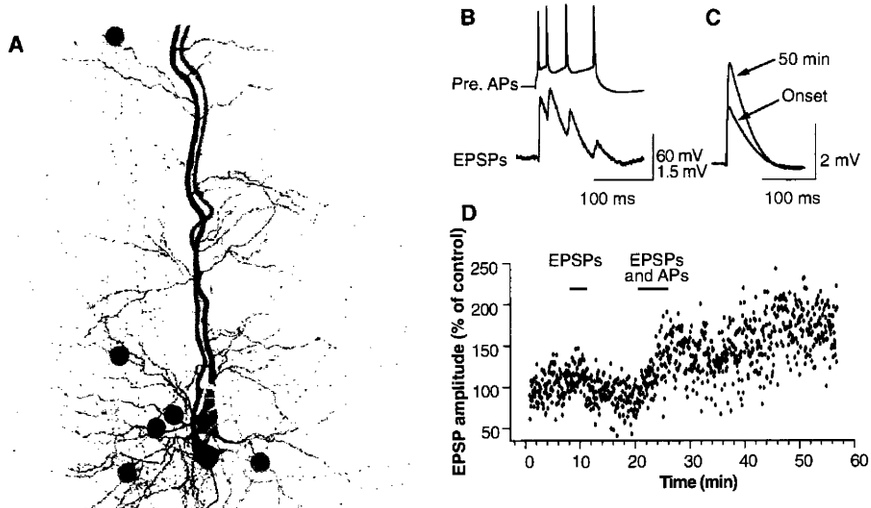

Fig. 1. Simultaneous pre- and postsynaptic activity in synaptically coupled neurons induces an increase in EPSPs. (**A**) Camera lucida reconstruction of a bidirectionally coupled pair of thick-tufted layer-5 pyramidal neurons. Putative synaptic contacts are marked by green dots (from the black neuron; five contacts; mean distance from soma, 95 μm; range, 73 to 126 μm) and blue dots (from the red neuron; six contacts; mean distance from soma, 95 μm; range, 50 to 283 μm). Thin dotted lines represent axon collaterals (blue is for the cell drawn in red; green is for the cell drawn in black). An average of 5.5 contacts are made per connection, and more than 80% of contacts are within 200 μm of the soma. (**B**) Characteristic synaptic response. A presynaptic burst of APs (Pre. APs) evoked by a 100-ms current pulse (400 pA, cell body injection) evokes EPSPs in the postsynaptic neuron. (**C**) Mean unitary EPSPs before and after pairing. Averages of 75 EPSPs from the onset and after 50 min. (**D**) Synchronization of pre- and postsynaptic activity. Each dot represents the amplitude of a single, test, AP-evoked EPSP shown as a percent of the average (of 75 responses, 5 min) control EPSP. Whole-cell recording was established about 3 min before time 0. After 10 min of recording, bursts of EPSPs were evoked 10 times every 20 s (indicated by the bar labeled EPSPs). Test EPSPs were continuously sampled every 4 s in between these bursts. After 20 min of recording, a burst of postsynaptic APs was evoked during EPSPs (15 times every 20 s; indicated by bar labeled EPSPs and APs).

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