

ed and used at 1:4 dilution in the IFN- $\gamma$  assay (17) in the presence or absence of anti-IGIF (25  $\mu$ g/ml) (6).

21. Wild-type or ICE-deficient mice were primed with *P. acnes* (20). Seven days later, mice were exposed to LPS (1  $\mu$ g, intravenously). In some experiments, recombinant mature IGIF (1  $\mu$ g) or protein G-purified anti-IGIF (250  $\mu$ g) was coinjected with LPS; sera were collected 3 hours after LPS exposure.
22. Reduced IFN- $\gamma$  was also observed in *Listeria*-infected (N. M. Tsuji *et al.*, in preparation) and LPS-exposed (G. Ku *et al.*, in preparation) ICE<sup>-/-</sup> mice.
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## A Synaptically Controlled, Associative Signal for Hebbian Plasticity in Hippocampal Neurons

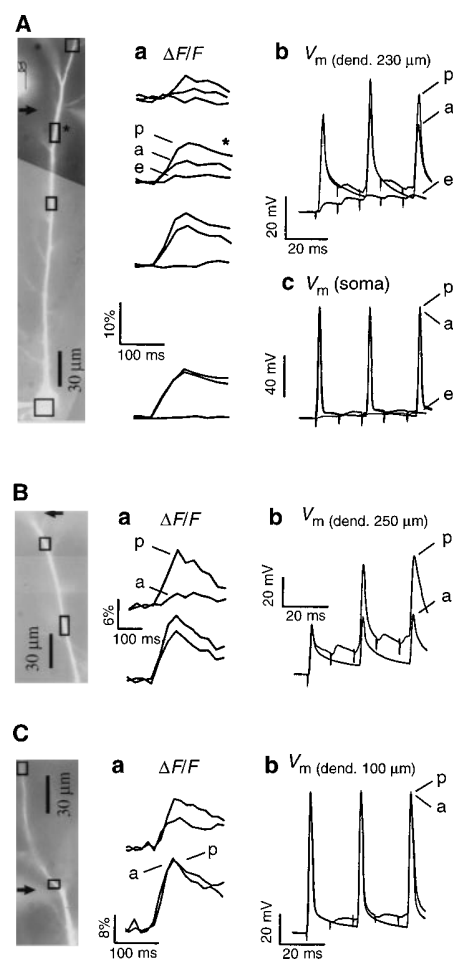
Jeffrey C. Magee and Daniel Johnston

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<sup>+</sup>, Ca<sup>2+</sup>, and K<sup>+</sup> 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) prop-

agate rapidly into the soma and dendrites, providing large membrane depolarizations and substantial increases in dendritic intracellular calcium ion concentration ([Ca<sup>2+</sup>]<sub>i</sub>) (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<sup>2+</sup> influx (Fig. 1) (11, 12). A subthreshold EPSP train produced a small and highly localized increase in [Ca<sup>2+</sup>]<sub>i</sub> (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<sup>2+</sup>]<sub>i</sub> (5%  $\Delta F/F$ ) (Fig. 1A). Pairing of synaptic stimulation and back-propagating action potentials, however, resulted in an increase in [Ca<sup>2+</sup>]<sub>i</sub> that was significantly larger than the simple sum of the two independent Ca<sup>2+</sup> signals (10%  $\Delta F/F$ ) (Fig. 1A). The amount of the pairing-induced increase in action potential amplitude and Ca<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup>]<sub>i</sub> in only the middle set of traces (\*). The supralinear increase in [Ca<sup>2+</sup>]<sub>i</sub> 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 non-regenerative. **(C)** Dual electrical recordings from a more proximal dendritic region. Pairing had little effect on Ca<sup>2+</sup> entry in lower box (Ca) or on action potential amplitude (Cb). In the more distal optical recording (upper box, 200  $\mu$ m), a larger increase in [Ca<sup>2+</sup>]<sub>i</sub> 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channels. In these regions, pairing EPSPs with action potentials increased action potential amplitudes by approximately twofold, whereas the associated increase in  $[\text{Ca}^{2+}]_i$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$  across the dendritic tree (9, 15). The occurrence of EPSPs during the back-propagation can thus modulate and sculpt the potential and  $\text{Ca}^{2+}$  influx into dendritic branches that receive the synaptic input.

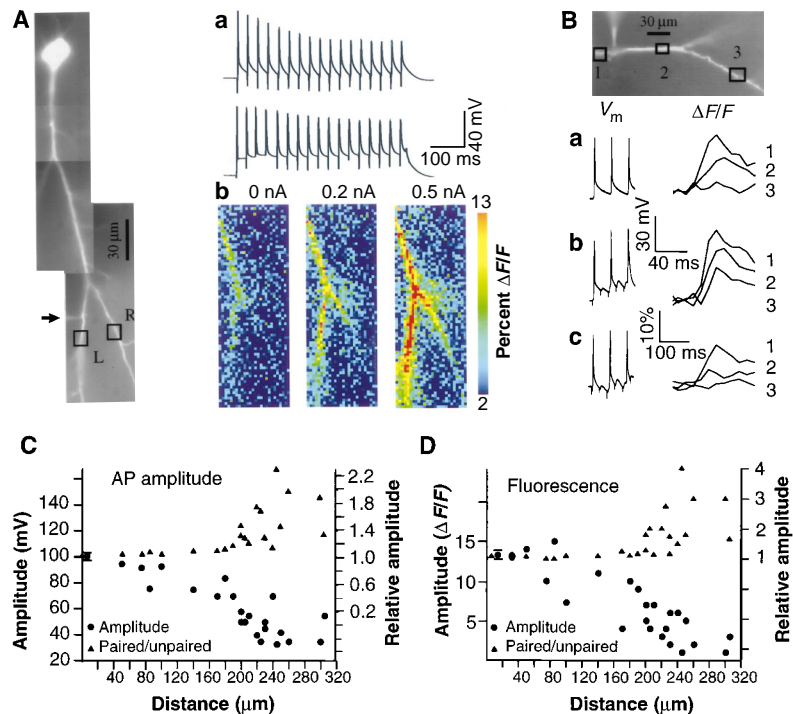
With the aid of simultaneous synaptic depolarization, back-propagating action potentials could provide the synaptic input region of a pyramidal neuron with a feedback signal that an output has occurred. Such a feedback signal is ideally suited for Hebbian modifications of synaptic strength (16). To test 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  $[\text{Ca}^{2+}]_i$  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  $[\text{Ca}^{2+}]_i$ , 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  $[\text{Ca}^{2+}]_i$  and induced significant LTP of the EPSPs (Fig. 3, D and E) (18).

The LTP was inhibited by  $\text{Ca}^{2+}$  channel antagonists nimodipine and  $\text{Ni}^{2+}$  (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  $\mu\text{M}$  failed to block LTP completely, the addition of 20  $\mu\text{M}$  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  $\mu\text{M}$  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  $[\text{Ca}^{2+}]_i$  were recorded in neuronal regions proximal to the TTX application (Fig. 4B). This local blockade of dendritic  $\text{Na}^+$  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  $\mu\text{m}$  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  $[\text{Ca}^{2+}]_i$  into both branches of the dendrite. **(B)** (Ba) Voltage and  $\text{Ca}^{2+}$  signals from the dendrite in response to unpaired action potential generation. There is no increase in  $[\text{Ca}^{2+}]_i$  in the dendrite distal to the major branch point located 260  $\mu\text{m}$  from the soma. (Bb) Pairing of EPSPs and action potentials increases action potential amplitude and rise in  $[\text{Ca}^{2+}]_i$ , 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 ( $\bullet$ ). The amplifying effect of paired stimulation is expressed as paired action potential amplitude divided by unpaired action potential amplitude ( $\blacktriangle$ ) and is also plotted as a function of distance from the cell body. **(D)** Plot of action potential-induced increase in  $[\text{Ca}^{2+}]_i$  as a function of distance from the cell body ( $\bullet$ ). The amplifying effect of paired stimulation on changes in  $[\text{Ca}^{2+}]_i$  is expressed as paired  $\Delta F/F$  divided by unpaired  $\Delta F/F$  ( $\blacktriangle$ ) and is also plotted as a function of distance from the cell body.

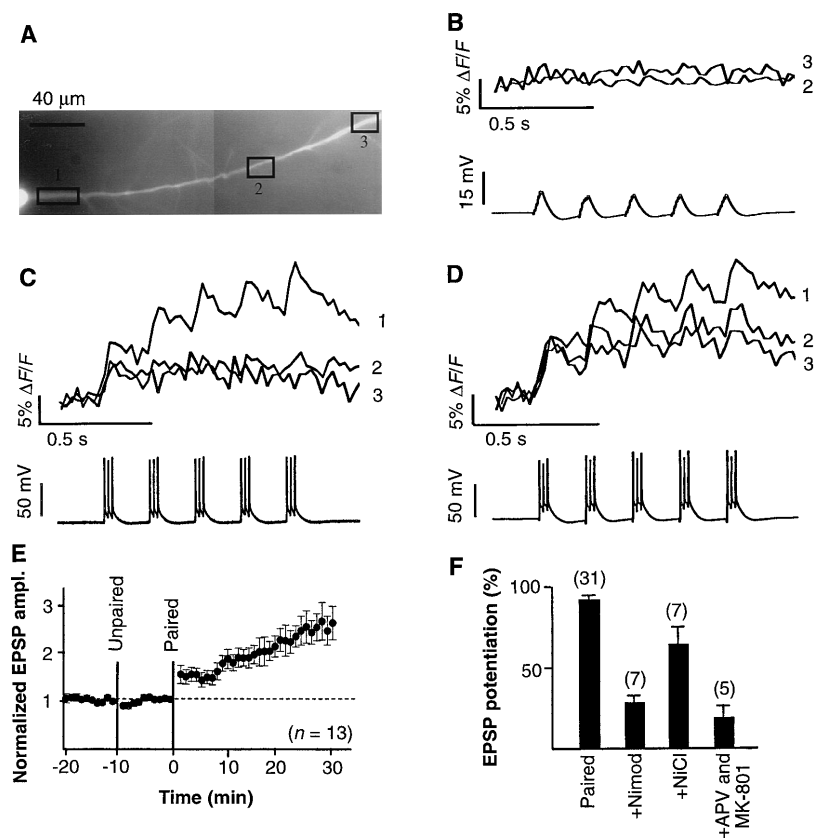
4F) (23). This result demonstrates the importance of the action potential amplification by synaptic depolarization, without which the amplitude of back-propagating

action potentials becomes insufficient to evoke both a large influx of  $Ca^{2+}$  and LTP in the more distal dendritic regions.

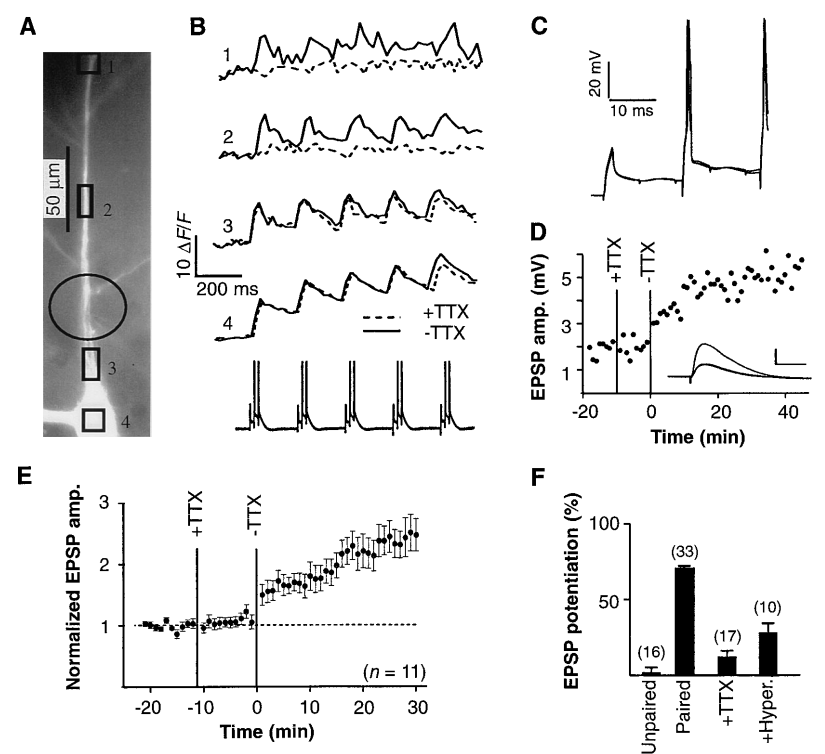
Using two different techniques to inhibit

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  $\mu$ M APV + 20  $\mu$ M MK-801, 10  $\mu$ M nimodipine, or 50  $\mu$ M  $Ni^{2+}$ . 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  $\mu$ M 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.







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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.

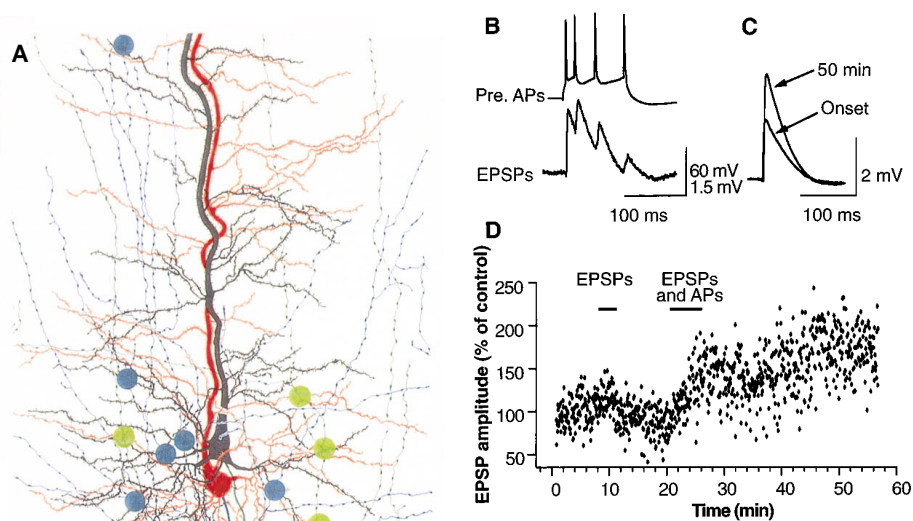
Repetitive activation of neuronal circuits can induce long-term changes in subsequent responses generated by synapses in many regions of the brain, and such plasticity of synaptic connections is regarded as a cellular basis for developmental and learning-related changes in the central nervous system (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 ampli-

tudes 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;  $94 \pm 23\%$  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 ( $38 \pm 9\%$ ;  $n = 21$ ; 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,  $95 \mu\text{m}$ ; range,  $73$  to  $126 \mu\text{m}$ ) and blue dots (from the red neuron; six contacts; mean distance from soma,  $95 \mu\text{m}$ ; range,  $50$  to  $283 \mu\text{m}$ ). 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  $200 \mu\text{m}$  of the soma. **(B)** Characteristic synaptic response. A presynaptic burst of APs (Pre. APs) 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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