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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

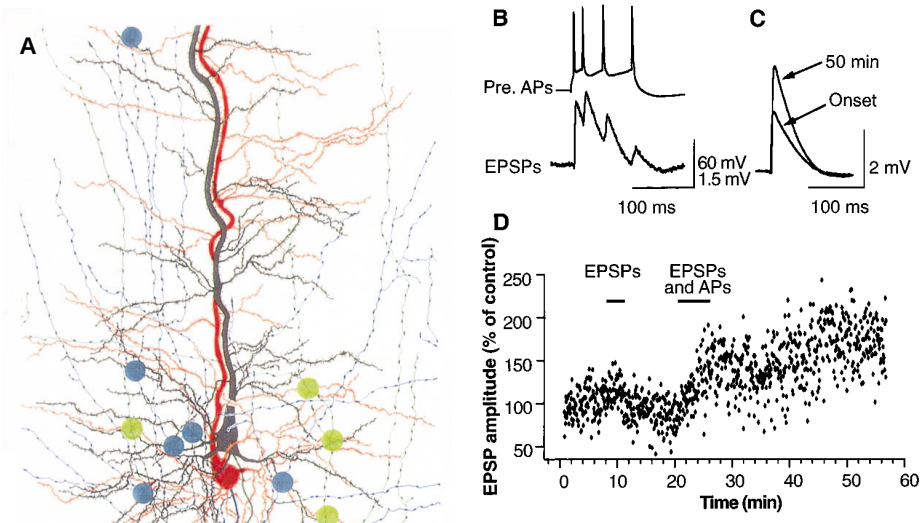

Fig. 1. Simultaneous pre- and postsynaptic activity in synaptically coupled neurons induces an increase in EPSPs. (**A**) Camera lucida reconstruction of a bidirectionally coupled pair of thick-tufted layer-5 pyramidal neurons. Putative synaptic contacts are marked by green dots (from the black neuron; 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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EPSPs was also dependent on the frequency of AP-EPSP pairs in the train, with a sharp onset at 10 Hz (Fig. 2C). Although APs paired with EPSPs reliably induced an increase in EPSP amplitude, pairing of sustained depolarization of the postsynaptic neuron to below the threshold for AP initiation or to between −30 and −10 mV (in the absence of AP initiation) with a high-frequency train of EPSPs failed to cause a significant change in EPSP amplitudes (Fig. 2D) (*10*), which suggests that initiation of postsynaptic APs and their coincidence with EPSPs was required to induce persistent changes in active synapses. Because most synaptic contacts were located on dendrites around 100 μm away from the cell body (Fig. 1A) (*7*), the coincidence of the back-propagating AP with active synapses (*11*) seems to be a critical step for induction of changes in EPSPs. The persistent increase in EPSPs caused by pairing of individual APs and EPSPs was prevented when *N*-methyl-D-aspartate (NMDA) receptors were blocked (*12*), which suggests that the back-propagating AP interacts with activated NMDA receptors to trigger the synaptic modification, for example, through Ca^{2+} inflow.

The limits of the time window for AP-EPSP interaction were investigated in uni- and bidirectionally connected neurons (*13*). When postsynaptic APs preceded EPSPs by 100 ms (Fig. 3, A and C) or followed the last EPSP in a burst by 100 ms (Fig. 3, A and C), no effect on EPSP amplitudes was observed, which suggests that APs and EPSPs must coincide within 100 ms in order to induce changes in EPSPs. To determine the limits of the AP-EPSP coincidence window, the effect of an AP initiated shortly before or shortly after the onset of an EPSP was tested simultaneously in bidirectionally connected neurons (Fig. 3B). A burst of APs was triggered at a frequency of 10 Hz in one cell; with a 10-ms delay, an identical burst was triggered in the other cell. This ensured that each postsynaptic AP in the burst occurred

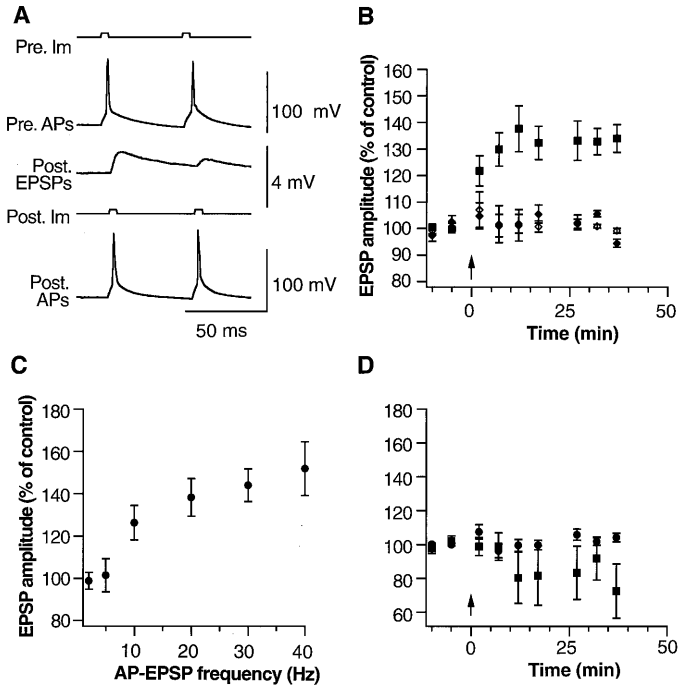

Fig. 2. AP-EPSP coincidence requirement. (**A**) Synchronization of individual postsynaptic APs with EPSPs. Two APs, separated by 50 ms, were evoked in a presynaptic neuron (Pre. APs), which elicited unitary EPSPs in the postsynaptic neuron (Post. EPSPs). Postsynaptic APs (Post. APs) were synchronized with each EPSP by injection of current pulses into the postsynaptic neuron (approximately 5 ms after the presynaptic AP). APs were evoked by injection of 5-ms, 1-nA current pulses into cell bodies (Im). (**B**) AP-EPSP coincidence. Three stimulation protocols were used. (i) Two, 5, or 10 EPSPs and postsynaptic APs at 20 Hz, paired 10 times every 4 s (n = 21; solid squares); (ii) 5 or 10 postsynaptic APs alone at 20 Hz (n = 5; open diamonds); and (iii) 5 or 10 EPSPs alone at 20 Hz (n = 5; solid diamonds). Time axes are normalized to the onset of the pairing (arrow at time 0). (**C**) Frequency dependence. Five EPSPs and postsynaptic APs at 2 (n = 2), 5 (n = 5), 10 (n = 9), 20 (n = 11), 30 (n = 3), and 40 Hz (n = 4) were synchronized in bursts and repeated 10 times every 4 s. (**D**) Sustained postsynaptic depolarization. Two stimulation protocols were used. (i) Circles represent eight experiments in which bursts of 5 to 10 EPSPs (at 20 to 40 Hz, repeated 10 times every 4 or 20 s) were synchronized with sustained depolarization to below the AP threshold (200 to 500 ms; −50 mV). (ii) Squares represent six experiments in which similar bursts were synchronized with depolarizations to between −30 and −10 mV in neurons loaded with Lidocaine [*N*-ethylbromide quaternary salt (QX-314, 5 mM; RBI, Bethesda, Maryland)].

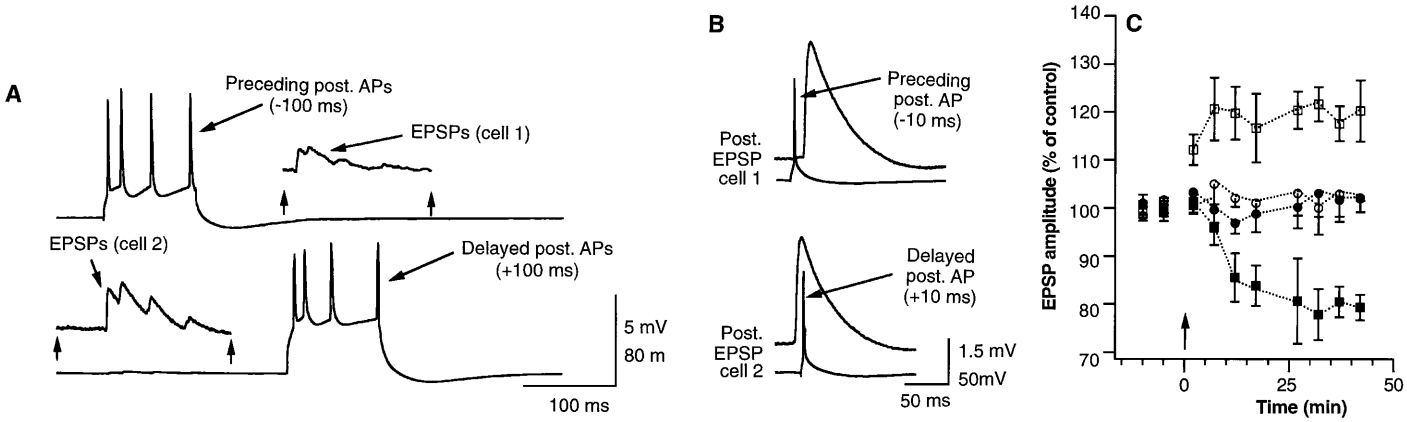

Fig. 3. Interaction and coincidence intervals. (**A**) Interaction interval. Bursts of postsynaptic APs were evoked in two bidirectionally coupled cells 100 ms apart, thus the interval of +100 ms and −100 ms between EPSPs and postsynaptic APs was investigated in the same slice and at the same time. The EPSPs are represented by the vertically expanded traces between the time points indicated by the arrows. Vertically expanded EPSPs of cell 1 were corrected for the skewed baseline. (**B**) Coincidence interval. A 10-Hz train of 5 APs in cell 1 (only first AP shown) was shifted by 10 ms in cell 2. An AP was thus triggered in cell 1 10 ms before an AP in cell 2 in a bidirectionally connected pair of neurons. This resulted in the postsynaptic APs occurring about 10 ms before the onset of the EPSP in cell 1 [−10 ms; solid squares in (C)] and about 10 ms after the onset of EPSPs in cell 2 [+10 ms; open squares in (C)]. This pattern was evoked in a burst of five such temporally shifted APs, at 10 Hz, and repeated 10 to 15 times every 4 s. (**C**) Interaction and coincidence intervals. Coincidence interval is represented by data from six bidirectionally coupled neurons. The averaged data when EPSPs and postsynaptic APs were 100 ms apart are also represented (+100 ms, open circles, n = 6; −100 ms, closed circles, n = 4).

either 10 ms before the onset of the EPSP in one cell and 10 ms after the onset of each EPSP in the other cell (Fig. 3, B and C). After this stimulation, the EPSP amplitude in the neuron in which the postsynaptic APs occurred 10 ms before the EPSPs was reduced, whereas it was increased when the postsynaptic AP occurred 10 ms after the onset of the EPSPs (Fig. 3, B and C), which suggests that postsynaptic AP can up- or down-regulate the amplitude of EPSPs, depending on the precise timing of synapse activation.

These observations suggest that the dendritic APs are a trigger for modifications of the functional synaptic connectivity between neocortical pyramidal neurons. The result of synaptic integration, encoded within the back-propagating AP, is conveyed to dendrites, and hence the back-propagating AP could be regarded as a "binding signal" for active synaptic contacts. A single synaptic contact could therefore be modified in the context of the summed activity of all the contacts on the neuron. The finding that EPSPs were up- or down-regulated, depending on the precise timing of EPSPs with respect to APs, further suggests that single synapses are modified according to the temporal relations of the electrical activity of the neurons in a network (*14*).

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6. Slice preparation, electrical recordings using Nomarski optics, and anatomical reconstructions for the localization of synapses were as described (*7*). Rats (Wistar) aged 14 to 16 days were used because cortical synapses are most plastic at these ages [K. Fox, *Neuron* **15**, 485 (1995)]. Data are presented as the mean ± SEM. The significance of changes was determined with independent two-tailed *t* tests (significance, $P < 0.05$).
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8. H. Markram and M. Tsodyks [*Nature* **382**, 807 (1996)] showed that the increase caused by pairing of pre- and postsynaptic activity only applies when tested by low-frequency (<5 Hz) stimulation.
9. Passive membrane properties were measured immediately after whole-cell measurements. Control test EPSPs evoked by single presynaptic APs at 0.25 Hz were recorded from 2 to 3 min after whole-cell recording. After a 10- to 12-min control period, the pairing protocol (detailed in the figure legends) was initiated, test EPSPs were recorded for 10 to 15 min, and the passive membrane properties were measured again. Test EPSPs were then continuously recorded for 30 to 60 min. Amplitudes of EPSPs (average of 75) were measured as the difference between onset (5 to 10 ms before) and peak of the EPSP (1 to 2 ms). Pairs in which the control average EPSPs differed by more than 10% were discarded. The maximum deviation from the baseline at any time during a 10- to 50-min period after pairing was used to represent the change in the EPSP. The time axes of all experiments were normalized to the pairing period.
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11. The conduction velocity in thin basal dendrites is estimated to be roughly 0.2 m/s [estimated from (*4*)] and the back-propagating AP would thus reach most synapses within 1 ms.
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Microtubule Treadmilling in Vivo

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In vivo, cytoplasmic microtubules are nucleated and anchored by their minus ends at the centrosome and are believed to turn over by a mechanism termed dynamic instability: depolymerization and repolymerization at their plus ends. In cytoplasmic fragments of fish melanophores, microtubules were shown to detach from their nucleation site and depolymerize from their minus ends. Free microtubules moved toward the periphery by treadmilling—growth at one end and shortening from the opposite end. Frequent release from nucleation sites may be a general property of centrosomes and permit a minus-end mechanism of microtubule turnover and treadmilling.

Microtubules (MTs) are fibrillar intracellular structures that play important roles in multiple cellular activities, including mitosis, transport, positioning of membrane organelles, and determination of cellular shape. MT arrays within cells are capable of rapid rearrangement that depends to a large extent on MT dynamics—the ability to exchange subunits between the soluble and polymer pools (*1*). Studies on MT dynamics in vitro demonstrate the existence of two principal mechanisms of subunit exchange known as treadmilling (*2*) and dynamic instability (*3*). Treadmilling involves the addition of subunits to one (plus) end of an MT and loss of subunits from the opposite (minus) end. Dynamic instability is defined by gain and loss of subunits at the same end (either plus or minus) of an MT during growth and shortening. In living cells, where the minus ends of MTs are believed to be tightly anchored at the centrosome, MTs are thought to exchange subunits by polymerization and depolymerization at their plus ends, thus using the dynamic instability mechanism (*4*).

We studied microtubule dynamics in cytoplasmic fragments of fish melanophores, which translocate cytoplasmic pigment granules to the center (aggregation) or to the periphery (dispersion) along a radial array of MTs (*5*). Remarkably, melanophore fragments retained the ability to aggregate pigment and organize a radial MT array of correct polarity orientation (with the minus ends at the center) (*6*) in the apparent absence of the centrosome (*7*). To study MT dynamics, we fluorescently tagged MTs in melanophores by microinjection of labeled tubulin, microsurgically dissected fragments from the parental cells, and induced pigment aggregation and formation of the MT aster (*8*). Images of labeled MTs were then sequentially acquired in the living fragments at short time intervals (3 s) for extended periods (10 min) (*9*).

Playback of the image sequences revealed characteristic patterns of MT behavior. At any given time, about 80% of the MTs ($n = 1067$) appeared to be static, with one end at the pigment aggregate and the other at the plasma membrane. The other 20% of MTs showed dynamic behavior, either primarily growing (10.6%), primarily shortening (9.3%), or moving (~1%) (Fig. 1A). MTs showed only short length excursions at their free ends, as if dynamic instability was suppressed. MTs emerged from the pigment mass and grew toward the periphery (Fig. 1A, MT1), which indicates that the pigment aggregate had the capacity to nucleate MTs. The aggregate also seemed to stochastically release MTs, after which they shortened at their proximal ends (Fig. 1A, MT2). Thus, the two populations of

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