virtue of their known activity against plasminogen activators, their role as tumor suppressors in breast cancer is not established (20). Similarly, the activity of metalloprotease inhibitors in breast cancer is not yet known (21).

From a clinical perspective, maspin offers substantial opportunities. First, the loss of expression that occurs during malignant progression of primary tumors suggests that maspin has potential value as a marker of a favorable prognosis. Maspin may also have therapeutic potential. The maspin gene is not lost in tumor cells but rather is down-regulated, as shown by the partial loss of expression in primary carcinomas and by its up-regulation in tumor cells after treatment with a phorbol ester (9). Maspin offers the potential of novel pharmacological approaches to therapy, such as inducing re-expression of the protein in breast cancers or blocking expression of the target protease.

REFERENCES AND NOTES

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Visualization of Quantal Synaptic Transmission by Dendritic Calcium Imaging

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As changes in synaptic strength are thought to be critical for learning and memory, it would be useful to monitor the activity of individual identified synapses on mammalian central neurons. Calcium imaging of cortical neurons grown in primary culture was used to visualize the activation of individual postsynaptic elements by miniature excitatory synaptic currents elicited by spontaneous quantal release. This approach revealed that the probability of spontaneous activity differed among synapses on the same dendrite. Furthermore, synapses that undergo changes in activity induced by glutamate or phorbol ester treatment were identified.

MINIATURE EXCITATORY SYNAPTIC CURRENTS (MESCs) ARE A FUNDAMENTAL FORM OF NEURONAL COMMUNICATION PRODUCED BY THE SPONTANEOUS RELEASE OF A SINGLE TRANSMITTER QUANTUM (1). EXPERIMENTS SUGGEST THAT AT CENTRAL SYNAPSES A SINGLE MESC IS EQUIVALENT TO A POSTSYNAPTIC RESPONSE RESULTING FROM THE EVOKED ACTIVATION OF A SINGLE SYNAPTIC TERMINAL (2). BY ANALOGY WITH QUANTAL ANALYSIS USED TO DEFINE MECHANISMS OF SYNAPTIC PLASTICITY AT NEUROMUSCULAR JUNCTIONS, ANALYSIS OF MESCs HAS BEEN USED TO PROVIDE INFORMATION ABOUT CHANGES IN RELEASE PROBABILITY AND POSTSYNAPTIC RESPONSIVENESS IN MODELS OF PLASTICITY IN BRAIN NEURONS (1). PROMPTED BY CONCERNS THAT ASSUMPTIONS IMPLICIT IN THESE ANALYSES MAY NOT BE APPLICABLE TO POPULATIONS OF CENTRAL SYNAPSES (3), WE HAVE VISUALIZED POSTSYNAPTIC CALCIUM TRANSIENTS INDUCED BY MESCs TO MONITOR ACTIVITY AT INDIVIDUAL SYNAPSES.

For this approach we used rat cerebral cortical neurons grown in primary culture (4, 5) because they form functional synapses (4, 5) and exhibit elevations in [Ca2+]i (6) due to spontaneous release of a single transmitter quantum (4). Experiment suggests that at central synapses a single MESc is equivalent to a postsynaptic response resulting from the evoked activation of a single synaptic terminal (6). By analogy with quantal analysis used to define mechanisms of synaptic plasticity at neuromuscular junctions, analysis of MESCs has been used to provide information about changes in release probability and postsynaptic responsiveness in models of plasticity in brain neurons (1). Prompted by concerns that assumptions implicit in these analyses may not be applicable to populations of central synapses (3), we have visualized postsynaptic calcium transients induced by MESCs to monitor activity at individual synapses.
potential-dependent synaptic activity. The detection of relatively homogeneous [Ca\(^{2+}\)]\(_i\), transients over the entire dendritic segment during SCTs (Fig. 2A) (6) suggests that all postsynaptic sites are responsive.

To better understand the relation between MSCTs and SCTs, we compared their kinetics (Fig. 2B) and sensitivity to NMDA receptor blockade (6) and found that they are similar. These similarities suggested that, to a first approximation, the spatial summation of segment during SCTs (Fig. 2A) (6) suggests multiple MSCTs could underlie evoked synaptic responses in fine dendritic processes. For example, two MSCTs occurring in close temporal succession produced a relatively homogeneous rise in [Ca\(^{2+}\)]\(_i\), in the intervening segment (Fig. 1A).

Whole cell recordings made with the same solutions used in the imaging studies indicated the presence of MESCs (6 to 50 pA) (11, 12) that were dependent on Glu receptors, as local or bath application of the broad spectrum ionotropic Glu receptor antagonist kynurenate greatly reduced their amplitude (13). The mean frequency of these currents (2.1 ± 0.5 Hz) was within the range of estimated frequencies for MSCTs per neuron (1 to 5 Hz) (14).

In the course of these imaging studies, multiple MSCT events often occurred at the same dendritic site (Fig. 1). Statistical analysis of these repeated MSCTs indicated that they are not explained by random behavior (15). At a high probability site (denoted B in Fig. 1A), five of nine total MSCTs were observed (the probability of this occurring at any one site randomly is 0.003). A total of 26 different dendritic fields were examined in which 49 distinct MSCT sites were observed. Of these sites, 14 displayed more than one MSCT. For each of these 14 sites, we calculated the probability of the repeated events occurring randomly at that specific site (15). In 11 of the 14 cases, the probability of repeated events at these identified specific sites was less than 0.05, suggesting that these sites have a higher probability of spontaneous activity than others within the population. However, from our data it is not possible to determine whether the heterogeneous distribution of MSCTs is due to differences in the rate of spontaneous transmitter release (1, 16, 17) or in postsynaptic responsiveness (18).

The presence of synapses with a high probability of spontaneous activity could indicate that they had undergone activity-dependent plasticity. Because the cultures used in the experiments illustrated in Fig. 1 were routinely synthetically stimulated (picrotoxin-induced bursting) (5, 6) for at least 15 min, 0.5 hour, and 3 hours before

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Fig. 1. The [Ca\(^{2+}\)]\(_i\), transients associated with quantal release. (A) Images shown in each row reflect [Ca\(^{2+}\)]\(_i\), at the indicated times in a 10-s run. As shown in the top row, this dendrite exhibited three separate local increases in [Ca\(^{2+}\)]\(_i\), within the 10-s sampling period. The first transient is initiated at a spine (site F) at \(t = 4\) s. Over the next 1 s, the rise in [Ca\(^{2+}\)]\(_i\), spreads to involve ~10 \(\mu\)m of the dendrite and four to five other spines. Another MSCT is observed at site F at \(t = 7.3\) s. At \(t = 7.7\) s, an independent [Ca\(^{2+}\)]\(_i\), transient originated at site B. Over the next few seconds, the rise in [Ca\(^{2+}\)]\(_i\), overlaps in the segment connecting them. In the next 10-s sample shown in the bottom row (done ~10 min later), [Ca\(^{2+}\)]\(_i\), returned to basal concentrations before the appearance of two additional MSCTs at site B. The plane of focus was adjusted slightly for the second run, accounting for the structural differences between the two panels. Calibration = 5 \(\mu\)m; the small boxes are 1.8 \(\mu\)m\(^2\) or 63 pixels. (B) Plots of [Ca\(^{2+}\)]\(_i\), as a function of time are displayed for the run shown in the top row for eight sites indicated in the first image in (A). The dashed lines mark the beginning of MSCT events.

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Fig. 2. Synchronous and local synaptic activation of dendrites. (A) A single cortical neuron was injected with fura 2 as described in Fig. 1, and ratiometric imaging was done in a fine spiny dendritic process before (top) and during a burst of action potential–dependent spontaneous synchronous synaptic activity. In the absence of TTX, [Ca\(^{2+}\)]\(_i\), rose relatively homogeneously in fine dendrites of all neurons examined \((n = 5)\). Calibration = 5 \(\mu\)m. (B) Comparison of MSCTs with synaptic [Ca\(^{2+}\)]\(_i\), transients (SCTs). The [Ca\(^{2+}\)]\(_i\), imaging was done in a fine spiny dendritic process during action potential–dependent synaptic activity (SCT) and during miniature synaptic activity (MSCT). Three [Ca\(^{2+}\)]\(_i\), transients were recorded under each condition, averaged, and normalized to a peak of 100%. Comparison of their time courses indicated that SCTs (control) and MSCTs (TTX) have similar kinetics.
Fig. 3. Location of a high probability synapse induced by Glu treatment. A 360-nm fura 2 image of a cortical neuron dendrite is shown. The neuron was kept under quiescent conditions for ~3 hours before membrane recordings were made. Four control runs of 10-s duration were made in which six MSCTs were observed at six different sites. The approximate boundaries of their [Ca\(^{2+}\)] transients are shown by boxes. Approximately 2 min after the last control run, the dendrite was exposed to 20-50 pulses of Glu (20 μM; separated by 1 min, applied by pressure ejection with a locally placed pipette). The effect of Glu was confirmed by observation of a large [Ca\(^{2+}\)] transient that encompassed the whole dendrite. Four minutes after [Ca\(^{2+}\)], had returned to control concentrations, MSCTs were assessed at 90-s intervals (five 10-s runs were recorded). After Glu treatment seven of eight total MSCTs observed occurred at the same site (initiated at the spine's location). The probability of seven of eight events occurring at any one of six locations randomly (a conservative assumption, as greater than six sites are indicated by spines) was calculated to be 0.0003. In the absence of Glu stimulation, the frequency of MSCT events and the number of high probability sites within a dendritic field were stable over time. For example, in a neuron not treated with Glu, 14 MSCT events were observed with no apparent high probability sites (15) in eight runs; 30 min later, 15 MSCT events were observed without the appearance of high probability sites in the same number of sampling runs. Calibration = 5 μm.

REFERENCES AND NOTES

4. Cortical neurons and glia were dissociated from 17- to 18-day gestation rat fetuses, placed in culture, and allowed to mature for at least 17 to 26 days in vitro as described (5). Cortical neurons were impaled with 40- to 60-Mohm microelectrodes that were filled (tip only) with 10 mM fura 2 (K+ salt, Molecular Probes) in 200 mM KCl (6). Electrodes were then removed and the cells allowed to recover in the presence of TTX for about 2 to 3 hours before assessment of [Ca\(^{2+}\)]. Ratio-metric fura 2 imaging was then done at room temperature (6-6). Images are the average of four consecutive video frames. The averaged images were acquired every 333 ms. Thus, in a typical 10-s run, 30 images were collected. The following extracellular solution was used to isolate MESCs: 137 mM NaCl, 5.0 mM KCl, 5.0 mM CaCl\(_2\), 0.44 mM KH\(_2\)PO\(_4\), 0.34 mM Na\(_2\)HPO\(_4\), 10 mM Na-glucose, 1 mM NaHCO\(_3\), 1 mM NaSCN, 0.001 mM tetrodotoxin, 0.001 mM, and 22 mM glucose (pH 7.4 and 340 mosM). To facilitate the observation of MSCTs, we removed extracellular Mg\(^{2+}\), which mimics effects of depolarization on NMDA receptor function. Thus, under physiological conditions, if a neuron were depolarized by activity at distant synapses, coincident activity at a single synapse could then elicit local NMDA receptor-mediated Ca\(^{2+}\) influx that would be expected to have characteristics similar to the transients we observed in the absence of Mg\(^{2+}\).
Locally Distributed Synaptic Potentiation in the Hippocampus

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The long-lasting increase in synaptic strength known as long-term potentiation has been advanced as a potential physiological mechanism for many forms of both developmental and adult neuronal plasticity. In many models of plasticity, intercellular communication has been proposed to account for observations in which simultaneously active neurons are strengthened together. The data presented here indicate that long-term potentiation can be communicated between synapses on neighboring neurons by means of a diffusible messenger. This distributed potentiation provides a mechanism for the cooperative strengthening of proximal synapses and may underlie a variety of plastic processes in the nervous system.

Most models of neuronal development, learning, memory, and circuit reorganization include alterations of the strength of synaptic connections between neurons. These models suggest that communication occurs between like synapses, such that synapses that are coactive tend to function as a group (1). Long-term potentiation (LTP) the long-lasting increase in synaptic transmission that is induced by intense synaptic activity (2), has been advanced as a potential physiological mechanism for these forms of plasticity. Although LTP clearly results in enhanced synaptic transmission, it is less clear whether it possesses the properties necessary to mediate the intercellular communication inherent in most models of coactive strengthening. When LTP is selectively induced in one synaptic pathway, other synaptic inputs to the same cell do not undergo LTP (3). This input specificity has usually been interpreted to mean that potentiation cannot be communicated from one synapse to another.

During the induction of LTP, postsynaptic events such as N-methyl-D-aspartate (NMDA) receptor activation and Ca2+ influx have been suggested to lead to the generation of different diffusible signals (4), such as arachidonic acid (5), carbon monoxide (6), nitric oxide (NO) (7–9), and platelet-activating factor (10, 11). These signals have been proposed to mediate the synaptic enhancement of LTP (5–13). In theory, a diffusible messenger could act in a strictly retrograde manner, influencing only the synapses where it is generated, or it could diffuse to enhance the synapses of nearby neurons as well. Indeed, an LTP induction procedure, pairing postsynaptic depolarization of a single neuron with low-frequency stimulation of afferent fibers (4) decreases the action potential latency in both the unstimulated cell as well as nearby cells (15, 16).

To examine whether long-lasting synaptic potentiation could spread to nearby synapses in hippocampal slices, we made simultaneous intracellular recordings from two nearby CA1 pyramidal neurons and monitored the excitatory postsynaptic potentials (EPSPs) resulting from stimulation of Schaffer collaterals (17) (Fig. 1A). We detected no synaptic or electrical coupling...