Selective Induction of LTP and LTD by Postsynaptic $[Ca^{2+}]_i$ Elevation

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Shao-Nian Yang, Yun-Gui Tang and Robert S. Zucker. Selective Induction of LTP and LTD by Postsynaptic $[Ca^{2+}]_i$ Elevation. *J. Neurophysiol*. 81: 781–787, 1999. Long-term potentiation (LTP) and long-term depression (LTD), two prominent forms of synaptic plasticity at glutamatergic afferents to CA1 hippocampal pyramidal cells, are both triggered by the elevation of postsynaptic intracellular calcium concentration ($[Ca^{2+}]_i$). To understand how one signaling molecule can be responsible for triggering two opposing forms of synaptic modulation, different postsynaptic $[Ca^{2+}]_i$ elevation patterns were generated by a new caged calcium compound nitrophenyl-ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid in CA1 pyramidal cells. We found that specific patterns of $[Ca^{2+}]_i$ elevation selectively activate LTP or LTD. In particular, only LTP was triggered by a brief increase of $[Ca^{2+}]_i$ with relatively high magnitude, which mimics the $[Ca^{2+}]_i$ rise during electrical stimulation typically used to induce LTP. In contrast, a prolonged modest rise of $[Ca^{2+}]_i$ reliably induced LTD. An important implication of the results is that both the amplitude and the duration of an intracellular chemical signal can carry significant biological information.

INTRODUCTION

Long-term potentiation (LTP) and depression (LTD) are two forms of activity-dependent synaptic plasticity that are broadly assumed to be involved in learning and memory (Bliss and Collingridge 1993; Linden and Conner 1995). In LTP, brief high-frequency afferent activity leads to a long-lasting increase in the strength of synaptic transmission, whereas prolonged low-frequency activity results in a persistent reduction in synaptic strength. Both processes are triggered by an increase in the level of postsynaptic intracellular calcium concentration ($[Ca^{2+}]_i$). Proposals that LTD is induced simply by a lower minimum $[Ca^{2+}]_i$ than LTP (the "differential threshold hypothesis") (see Artola and Singer 1993; Lisman 1989) are not supported by experiments showing that LTD and LTP have similar probabilities of being activated by a brief rise in postsynaptic $[Ca^{2+}]_i$, in the submicromolar range (Neveu and Zucker 1996a). One possibility is that a rise in $[Ca^{2+}]_i$ enables the induction of both LTD and LTP but that other processes activated by the different patterns of afferent stimulation are responsible for determining the outcome, for example, activation of muscarinic (Huerta and Lisman 1995), metabotropic (Bortolotto et al. 1994; Cohen and Abraham 1996), or *N*-methyl-D-aspartate (NMDA) (Huang et al. 1992) receptors. Another possibility is that LTP and LTD are specifically activated by different patterns of $[Ca^{2+}]_i$ elevation.

Previous studies (Neveu and Zucker 1996a,b) with photosensitive Ca^{2+} chelators to induce LTP or LTD were severely constrained by the limitations of available chelators. Nitr-5 changes its Ca^{2+} affinity by only 30-fold so that $[Ca^{2+}]_i$ cannot be elevated to a very high level. Even moderate increases in $[Ca^{2+}]_i$ require nearly complete photolysis of nitr-5 so that repeated photolysis cannot be used to achieve a prolonged rise in $[Ca^{2+}]_i$. Photolysis of DM-nitrophen produces a large $[Ca^{2+}]_i$ rise, and repeated partial photolysis can be used to prolong a small $[Ca^{2+}]_i$ rise, but interaction of this substance with endogenous cytoplasmic magnesium leads to a rise in $[Ca^{2+}]_i$ while filling the cell (the "loading transient") (Neher and Zucker 1993), which can itself induce either LTP or LTD even before beginning the recording session or photolysing the compound. In this study, we exploit the properties of the recently introduced chelator nitrophenyl- ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) (Ellis-Davies and Kaplan 1994) to produce either a large rise in $[Ca^{2+}]_i$ with nearly full photolysis or a prolonged rise in $[Ca^{2+}]_i$ with repeated partial photolysis without binding magnesium ions and the complication of a loading transient. This allows one to manipulate $[Ca^{2+}]_i$ levels in ways that were not previously possible.

We used selected patterns of photolysis of nitrophenyl-EGTA to show that, when the amplitudes and durations of $[Ca^{2+}]_i$ elevation are adjusted to resemble those occurring during electrical stimulation used to induce LTP or LTD (Otani and Connor 1996; Petrozzino et al. 1995), LTP or LTD is selectively and exclusively produced by such changes in $[Ca^{2+}]_i$.

METHODS

Transverse hippocampal slices, 400 μm thick, were cut by vibratome from brains of 11- to 22-day-old Sprague-Dawley rats after halothane anesthesia and decapitation (Neveu and Zucker 1996a). Slices were maintained ≥1 h in recording medium containing (in mM) 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 D-glucose, saturated with 95% O_2-5% CO_2. They were then transferred to a submersion chamber and perfused continuously at 2.5 ml/min at 22°C. Whole cell recording electrodes (4–7 MΩ) were filled with (in mM) 117.5 Cs gluconate, 17.5 CsCl, 8 NaCl, 10 N-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 0.2 EGTA, 2 Na_2ATP, and 0.2 GTP, pH 7.2 adjusted with CsOH. Electrode tips were filled with this solution containing 5–8 mM nitrophenyl-EGTA (Molecular Probes), 50% loaded with Ca^{2+}, and neutralized with 7.5–12 mM HCl. Excitatory postsynaptic potentials (EPSPs) were recorded under current clamp, holding cells at a membrane potential of −80 mV, after correction for a −6 mV junction potential, determined by measuring the potential difference between recording medium and pipette solution with 3 M KCl microelectrodes and a KCl

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agar bridge as ground lead. We continuously monitored pipette access and cell input resistances by recording responses to a square pulse of hyperpolarizing current (usually 30 pA) and balancing the initial access resistance with a bridge circuit. Access resistance ranged between 20 and 80 MΩ and varied <20% during experiments. Field recordings were made in the stratum radiatum with a glass microelectrode (3–6 MΩ) filled with 3 M NaCl. Schaffer collateral/commissural afferents were stimulated in the same layer with bipolar stainless steel electrodes by using 100-μs pulse of 0.5 to 5 V. Stimulus electrodes were positioned to excite afferents probably ending ≤150 μm of the postsynaptically recorded cell body, as judged by the rapid rise time of postsynaptic responses and from electrotonic calculations (Tsai et al. 1994). Such proximal synapses are more likely to be fully equilibrated with the pipette solution when recordings are made 20 min after forming seals and breaking into cells. Large responses contaminated with active membrane responses were avoided. Responses were sampled at 10 kHz, and maximal rising slopes were calculated on-line with the Neurophysiologia program (David Selig, San Francisco) by using a least-squares regression to cubic spline curve fits. In field recordings, postsynaptic responses were distinguished from electrical artifacts and presynaptic signals by observing the recruitment of a delayed negative potential after a graded artifact as stimulus intensity was increased. Because stimulation was optimized for minimal excitation of proximal synapses in the recorded cell and extracellular electrodes were not repositioned after establishing whole cell recording, field potentials often had very small postsynaptic components. The maximum slope of the rising phase of this component after the artifact returned to baseline was measured. Responses were normalized to average values 5–10 min before light exposure, and every other response is plotted to enhance clarity. In summary graphs, responses are aligned with respect to light exposures (Fig. 2) or electrical stimulation (Fig. 3), averaged across experiments in 40-s bins, and means plotted ± SE. Significance was evaluated with Student's *t*-test (two-tailed), comparing all points before photolysis to all points afterward.

Photolysis was accomplished with a standard 100-W Hg lamp (Nikon) fitted with a Uniblitz (Vincent Associates) shutter and aimed directly at the slice, beginning at ≥20 min after starting perfusion of cells with photolabile Ca^{2+} chelator from patch pipettes. In LTP-inducing experiments, an *f*/1.5 75 mm focal length quartz lens was used to increase light intensity. The light intensities with and without the lens (1.0 and 0.13 W/cm^2, respectively) were measured with a radiometer (International Light Model IL1700) by using a filter (Schott glass UG-1) with a transmittance spectrum similar to the excitation spectrum of nitrophenyl EGTA, after correcting for the reduction in intensity caused by the UG-1. Infrared and far-ultraviolet radiation was minimized with a liquid filter (Landò and Zucker 1989) to minimize cytotoxic and heating effects of light. Photolysis rate was measured in microcuvettes (with DM-nitrophen as described by Zucker 1994, and converting to nitrophenyl EGTA from the known relative quantum efficiencies and absorbances at 360 nm of the two photolabile chelators) at 87%/s with the lens and 11%/s without it.

In calculations of postsynaptic $[Ca^{2+}]_i$, the light intensity was assumed to be reduced by a factor of 2 to account for absorbance of light at a depth of 100 μm in the slice, based on our measurements of light absorbance at 360 nm in cortical slices of varying thickness (360 nm is the center of the action spectrum for photolyzing nitrophenyl-EGTA). Effects of photolysis on $[Ca^{2+}]_i$ were estimated from a model of nitrophenyl-EGTA photolysis and binding kinetics in cells. The model, written in BASIC programming language for Intel-based personal computers, is similar to models that were previously described in some detail (Landò and Zucker 1989; Zucker 1994). It is a single-compartment model, appropriate for uniform full-field illumination of a small cell where intracellular gradients in light intensity caused by self-absorption are minimal. The model includes the binding of Ca^{2+} to nitrophenyl-EGTA and its photoproduct and the effect of light on the respective levels of photolyzed and unphotolyzed nitrophenyl-EGTA (Ellis-Davies et al. 1996), the binding of Ca^{2+} to native buffers, assumed to be present at 1 mM concentration with 10-μM affinity and 20 mM · ms binding rate, and Ca^{2+} extrusion by a first order pump, with pump rate (0.1 ms^{-1}) adjusted to remove Ca^{2+} with a time constant of 1 s without additional exogenous buffer (Regehr and Tank 1992). A commented program listing and program disk are available from the authors on request.

Given the 20–30 min we allow for equilibration of intracellular and pipette solutions, nitrophenyl-EGTA concentration was assumed to equal that of the patch pipette, for the following reasons. Filling of the soma from 20- to 80-MΩ pipettes should occur with a time constant of from 1.8 to 7.4 min for access resistances of 20–80 MΩ (Eq. 18 in Pusch and Neher 1988); equilibration of the first third of 500-μm dendrites (Tsai et al. 1994), where the EPSPs we measure are likely to arise, should occur with a characteristic time of ~1 min ($x^2/4D$, x = 0.0015 cm, $D = 10^{-6}$ cm^2/s) (from Gabso et al. 1997); and fura-2 has been shown to reach 90% equilibration in proximal dendrites of pyramidal cells within 10 min (Helmchen et al. 1996). Nitrophenyl-EGTA purity was measured by Scatchard analysis at >95%. This information was used to adjust Ca^{2+} loading of the chelator to leave resting $[Ca^{2+}]_i$ undisturbed at 100 nM. Despite these precautions, significant uncertainties exist in the concentration, affinity, and kinetics of native buffer, concentration and Ca^{2+}-loading of nitrophenyl-EGTA, light intensity at cells recorded within a slice, and rate of Ca^{2+} removal by ion pumps. Therefore simulations of the effects of nitrophenyl-EGTA photolysis on $[Ca^{2+}]_i$ remain only rough order-of-magnitude estimates.

RESULTS

According to the differential threshold hypothesis (Artola and Singer 1993; Lisman 1989), LTD has a low intrinsic $[Ca^{2+}]_i$ threshold and is selectively induced by a modest rise in $[Ca^{2+}]_i$, similar to what occurs during induction of LTD by low-frequency afferent stimulation. LTP is selectively induced by a greater rise in $[Ca^{2+}]_i$, exceeding its higher intrinsic $[Ca^{2+}]_i$ threshold, as occurs during high-frequency afferent stimulation. The problem with this hypothesis is illustrated in Fig. 1. Whole cell recordings were made from a CA1 pyramidal cell (Fig. 1, *top traces*) simultaneously with field recordings from the stratum radiatum (*middle traces*). The recorded pyramidal cell was perfused for 20 min with a partially calcium-loaded solution of the caged calcium compound nitrophenyl-EGTA (Ellis-Davis and Kaplan 1994). The slices were exposed to two light flashes that partially photolyzed the nitrophenyl-EGTA, calculated to elevate postsynaptic $[Ca^{2+}]_i$ by ~800 nM (Fig. 1, *bottom traces*). This treatment caused a long-lasting depression in afferent synaptic transmission to the cell containing nitrophenyl-EGTA in two slices (Fig. 1, *left panels*, 30.0 ± 4.8% reduction in rising slope of EPSP, mean ± SD), whereas in another experiment a long-lasting potentiation was produced (*right panels*, 41.6% increase in EPSP slope). Field potentials, dominated by cells not filled with nitrophenyl-EGTA, are unaffected by light exposures. When only a single light exposure was used, elevating $[Ca^{2+}]_i$ by only 130 nM, no effects on synaptic transmission were observed.

These results resemble more extensive results obtained with a different caged calcium compound, nitr-5 (Neveu and Zucker 1996a), in which similar short-lasting minimal threshold $[Ca^{2+}]_i$ elevations were found to be sufficient to induce LTP or LTD. In that study it was also shown that photolytic induction of long-lasting depression occluded the induction of LTD by low-frequency afferent stimulation, that photolytic induction of

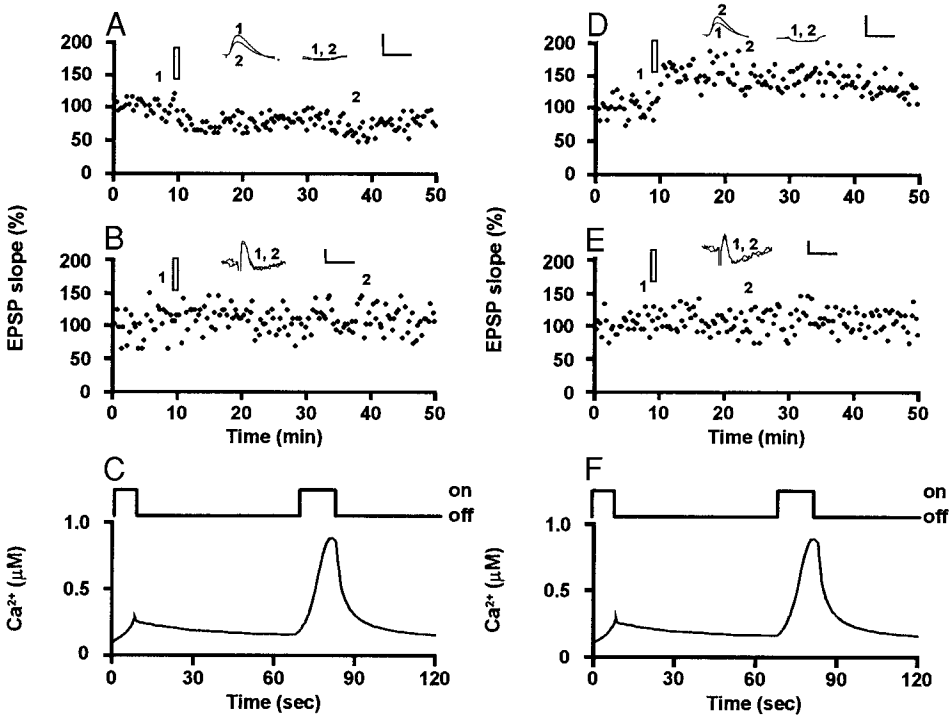

FIG. 1. A modest $[Ca^{2+}]_i$ elevation can induce either long-term depression (LTD) or potentiation (LTP). *Left* and *right panels* are from 2 different slices. *A* and *D*: whole cell recordings of excitatory postsynaptic potentials (EPSPs) to afferent stimuli repeated every 10 s; cells were filled with nitrophenyl-ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). *B* and *E*: simultaneous recordings of field potentials. In both cases, maximum slope of rising phase of EPSP is plotted vs. time. Insets show individual responses at times marked by numbers. *A* and *D* also include resistance measurements as responses to constant current pulses (−10 to −30 pA in all figures). Calibration bars: 10 mV and 50 ms (*A* and *D*, and in whole cell recordings in all figures); 0.2 mV and 10 ms (*B* and *E*, and in field potential recordings in all figures). Open vertical bars indicate photolysis by 8- and 15-s light exposures separated by 1 min. *C* and *F*: calculated (approximate) effects of the photolysis on $[Ca^{2+}]_i$ in recorded neurons are shown below time course of light exposures.

long-lasting potentiation occluded the induction of LTP by high-frequency stimulation, and that photolytically induced LTD and LTP shared pharmacological sensitivities to phosphatase and calmodulin inhibitors with electrically induced LTD and LTP respectively. Taken together, the results indicate that LTD and LTP can both be induced by brief submicromolar elevations in postsynaptic $[Ca^{2+}]_i$, even in the absence of presynaptic activity (Neveu and Zucker 1996b).

To determine whether LTD and LTP can be selectively activated by different temporal patterns of postsynaptic $[Ca^{2+}]_i$ elevation, we used different patterns of light exposure to try to mimic the changes in $[Ca^{2+}]_i$ that typically accompany induction of LTD and LTP by afferent stimulation (Otani and Connor 1996; Petrozzino et al. 1995). The main results are shown in Fig. 2. In the *left panels*, slices were exposed to repeated dim flashes of the same intensity as in Fig. 1, of durations from 2.6 to 15 s, spaced over a 45-s interval, and designed to produce a rise in $[Ca^{2+}]_i$ to ~750 nM and lasting ~1 min. The photolysis light pattern is shown in Fig. 2*E*, *top*. The calculated temporal profile of $[Ca^{2+}]_i$ elevation shown in Fig. 2*E*, *bottom*, is similar in magnitude to that occurring during 1-Hz stimulation (Otani and Connor 1996; Petrozzino et al. 1995), and Mulkey and Malenka (1992) showed that as little as 30–60 s of such stimulation can induce measurable LTD. Effects of photolysis on EPSP rising slopes from a typical injected cell are shown in Fig. 2*A*, with field recordings in Fig. 2*B*. All of the responses from 20 to 30 min after photolysis were depressed below all of the responses before photolysis ($P \ll 0.0001$ by Wald-Wolfowitz 2-sample runs test), and a similar comparison in the field recordings shows little change ($P > 0.4$). The EPSP reduction was accompanied by no change in either pipette access or cell input resistance. Sample responses to current injection are shown in Fig. 2*A*.

In repetitions of this experiment, in which $[Ca^{2+}]_i$ was probably elevated to ~750 nM for ~1 min, LTD was induced, or synaptic transmission was persistently depressed, in five of six slices. The average reduction in EPSP slope in these five slices was 25.8 ± 13.9% (measured as a difference in all points before photolysis to all points afterward, $P < 0.02$), as illustrated in Fig. 2*C*. Averaged changes in field recordings from these slices (Fig. 2*D*) show no change (reduction of 11.1 ± 10.1%, $P > 0.05$). Photolysis-induced LTD often developed slowly, as reported for LTD induced by electrical stimulation (Cummings et al. 1996). LTP was never activated by this protocol. Control experiments show that repeated light exposures of this intensity and with durations ≤15 s have no effect on EPSPs in cells not filled with a photolabile chelator (see Fig. 1*D*) (Neveu and Zucker 1996a).

In contrast, Fig. 2, *right panels*, illustrate effects of single brighter exposures lasting 10 s that probably elevated $[Ca^{2+}]_i$ to >10 μM (Fig. 2*J*) but only for a few seconds (calculated half-height width of $[Ca^{2+}]_i$ rise is 2.5 s). In 10 of 28 slices, LTP, or a persistent enhancement of synaptic transmission, was induced shortly after photolysis of nitrophenyl-EGTA in the cell where $[Ca^{2+}]_i$ was elevated to higher levels by intense photolysis (49.3 ± 12.4% increase in EPSP slopes, comparing all responses before and after photolysis, $P < 0.001$, Fig. 2*H*). Fig. 2*F* plots responses from a typical cell showing LTP, and Fig. 2*G* presents simultaneous field recordings. There was no significant change (increase of 2.7 ± 10.9%, $P > 0.1$) in slices where EPSPs were potentiated in the recorded cell (Fig. 2*I*). Control experiments show that, when unfilled cells are exposed to the bright illumination used to rapidly photolyze nitrophenyl-EGTA, there was no effect on EPSP amplitude (Fig. 3*A*).

In the remaining 18 slices, photolysis induced no significant change in the recorded EPSP slope (7.7 ± 28.6% increase, $P > 0.05$) or the field EPSP slope (0.8 ± 10.8% increase, $P > 0.15$). Figure 3, *B* and *C*, shows the results from a typical cell and the averaged responses of the 18 nonresponding cells. These records also demonstrate the stability of our responses

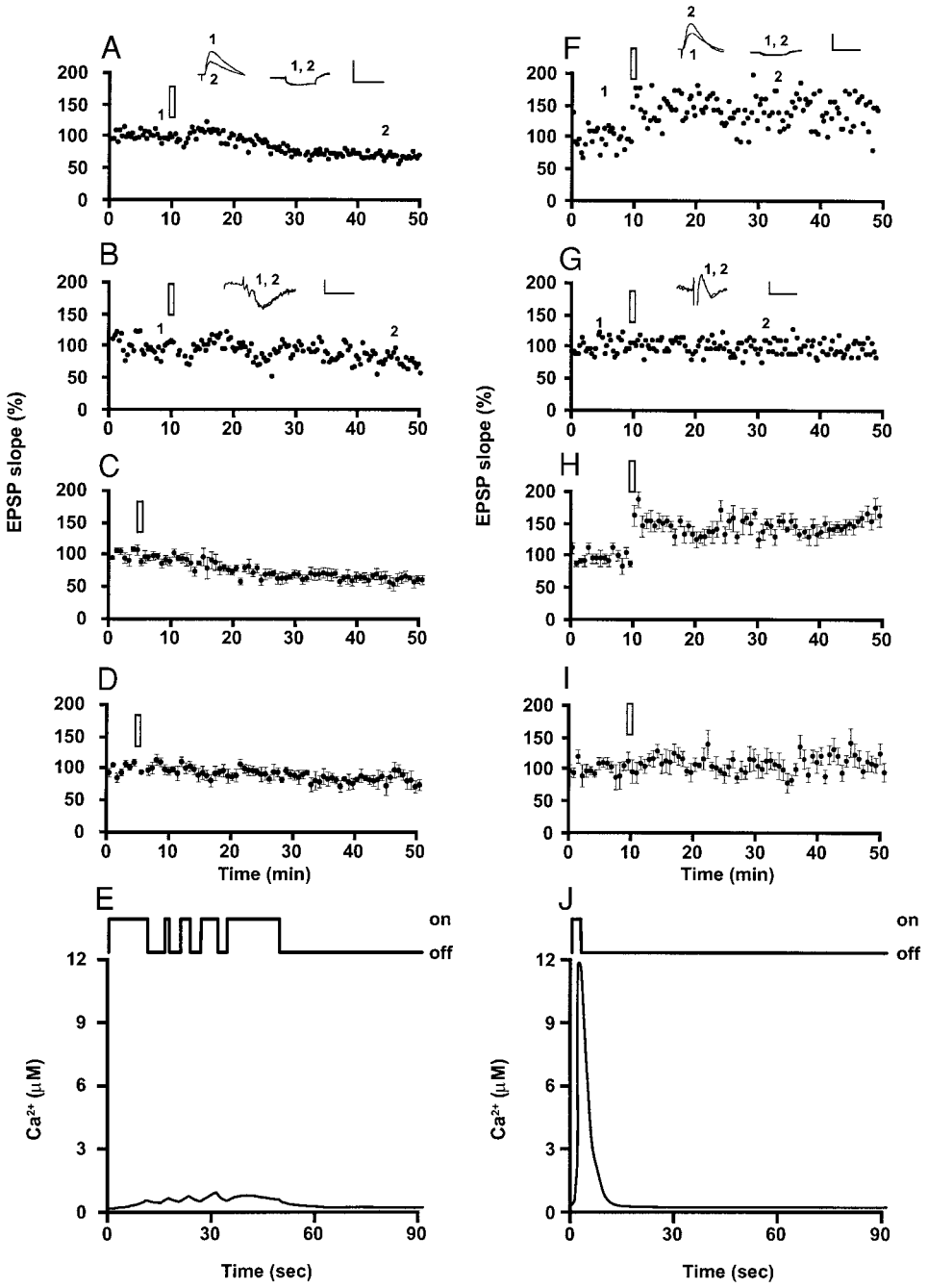

FIG. 2. Selective induction of LTD and LTP by specific patterns of $[Ca^{2+}]_i$ elevation. *Left* and *right panels* are from 2 different slices. *A* and *F*: whole cell recordings of EPSPs from neurons filled with nitrophenyl-EGTA. *B* and *G*: field potential recordings. Insets and calibration bars as in Fig. 1. *C*, *D*, *H*, and *I*: summary graphs of averaged intracellular EPSPs (*C* and *H*) and field potential recordings (*D* and *I*) from 5 (*C* and *D*) and 10 (*H* and *I*) slices in which long-lasting synaptic plasticity was induced. Vertical bars mark photolysis times. *E* and *J*: calculated (approximate) effects of photolysis on $[Ca^{2+}]_i$ by using different light exposure protocols to induce LTD and LTP. The pattern of light exposures is shown above the $[Ca^{2+}]_i$ traces. The light intensity in *F–J* was 8 times that used in *A–E* and in Fig. 1.

for prolonged recording periods and the absence of deleterious effects of photolysis photoproducts, confirming that the LTD generated by a more modest but prolonged $[Ca^{2+}]_i$ elevation (Fig. 2, *A* and *C*) is due neither to drift in baseline response amplitude nor to photoproduct toxicity. A clear instance of LTD was never activated by this intense light protocol. Apparently, LTD and LTP are selectively and exclusively activated by the two patterns of $[Ca^{2+}]_i$ elevation, LTD by a prolonged low increase in $[Ca^{2+}]_i$ and LTP by a brief larger increase in $[Ca^{2+}]_i$.

Although a prolonged modest rise in $[Ca^{2+}]_i$ almost always induced LTD, a brief intense rise in $[Ca^{2+}]_i$ produced what clearly appeared to resemble LTP, defined as a significant and persistent increase in EPSP in the absence of significant changes in field potential responses, in fewer than one-half the slices. This is not because the cells are incapable of exhibiting LTP. In seven of eight slices in which the LTP-inducing light exposure protocol failed to induce LTP ($3.6 \pm 8.3\%$ decrease, $P > 0.05$), a subsequent afferent tetanus (3 1-s trains, 100 Hz, with 1-s pauses) still generated LTP in the recorded cell ($65.6 \pm 21.6\%$, $P < 0.001$) as well as in the rest of the slice as indicated by the field recordings (Fig. 4, *left panels*). In contrast, in six slices in which nitrophenyl-EGTA photolysis did induce LTP ($65.9 \pm 23.6\%$ increase, $n = 6$, $P < 0.01$), subsequent tetanic afferent stimulation was without further effect ($2.0 \pm 15.1\%$ further increase, $P > 0.05$), except for a transient increase reflecting posttetanic potentiation (Fig. 4, *middle panels*). Moreover, like electrically induced LTP

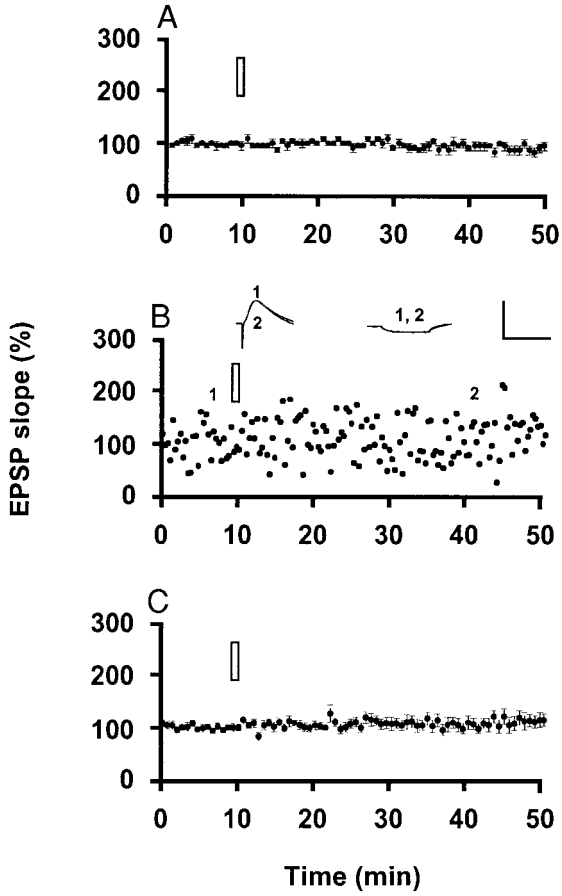

FIG. 3. Control experiments for effects of intense light and baseline drift. *A*: lack of effect of a bright light exposure (vertical bar) on EPSPs in a recorded cell perfused with a solution containing no nitrophenyl-EGTA. *B* and *C*: whole cell recordings from a single cell (*B*) and summary results from 18 slices (*C*) in which the bright light stimulus failed to influence EPSP amplitude. Insets and calibration bars as in Fig. 1.

(Mulkey and Malenka 1992), photolysis-induced LTP (52.6 ± 12.6% increase, $n = 6$, $P < 0.001$) could be reversed (to within −3.1 ± 9.8% of initial levels, $P > 0.05$) by subsequent low-frequency stimulation (1 Hz for 10 min; Fig. 4, *right panels*). Thus the long-lasting potentiation of synaptic transmission evoked by a brief strong $[Ca^{2+}]_i$ elevation in 36% of our experiments appears to be genuine LTP. We are unable to elevate $[Ca^{2+}]_i$ to high levels for more than ~2 s before exhaustion of nitrophenyl-EGTA, and it was previously found that $[Ca^{2+}]_i$ must remain elevated for >2 s to reliably induce LTP (Malenka et al. 1992). Even a 1-s tetanus at 100 Hz only evokes LTP if $[Ca^{2+}]_i$ is permitted to remain elevated for ~2–3 s. Therefore our LTP inducing protocol would be expected to induce LTP only some of the time, just as we observed. The important result is that it never induced LTD, whereas a prolonged small $[Ca^{2+}]_i$ increase almost always did.

The striking differences in effectiveness of the protocols illustrated in Fig. 2, *left* and *right panels*, in selectively inducing LTD and LTP are revealed by analysis of the results in the form of a two-way classification table. The LTD-inducing protocol reduced transmission in five cells and had no effect in one. The LTP-inducing protocol increased transmission in 10 cells and had no clear persistent effect in 18. These patterns differ at the $P \ll 0.0001$ level of significance ($\chi^2_2 = 27.5$).

DISCUSSION

When the results of Figs. 1 and 2 are considered together, they demonstrate that neither a difference in the amplitude of $[Ca^{2+}]_i$ elevation nor a difference in duration is sufficient by itself to determine the response of synapses to this chemical signal. A modest brief $[Ca^{2+}]_i$ elevation induces LTP or LTD with equal probability (Neveu and Zucker 1996a). Substantially increasing the amplitude of the $[Ca^{2+}]_i$ trigger selectively activates LTP, whereas substantially increasing the duration of this signal selectively activates LTD. Thus both amplitude and duration of the $[Ca^{2+}]_i$ signal are important in determining the neuron's response. Furthermore, the results show that both forms of synaptic plasticity can be reliably and selectively induced by different patterns of $[Ca^{2+}]_i$ elevation, without the need to invoke any other Ca^{2+}-independent signaling or triggering process. This is reminiscent of the differential activation of transcription factors by specific patterns of $[Ca^{2+}]_i$ elevation (Dolmetsch et al. 1997).

It might be thought that the variable responses to a brief modest $[Ca^{2+}]_i$ elevation are due to a spatial nonuniformity in $[Ca^{2+}]_i$ in response to modest photolysis. We do not deny that the elevation in $[Ca^{2+}]_i$ is likely to be higher in more superficial dendrites than in deeper processes. Nevertheless, the straightforward differential minimal threshold hypothesis predicts that, as the level of photolysis is gradually increased, LTD induction will always be observed first. This is the result we originally expected, and it is in particular exactly what we did not observe (Neveu and Zucker 1996a).

Likewise, global spatial $[Ca^{2+}]_i$ nonuniformity across the entire dendritic tree is likely to be present during the intense photolysis that elicits only LTP and to prolonged weak photolysis that induces only LTD. However, within the restricted regions of a cell activated by one or a few neighboring inputs, there is likely to be little variation in local light intensity and the degree of photolysis and hence little variation in the rise in $[Ca^{2+}]_i$. If such variation were a prominent feature of the $[Ca^{2+}]_i$ rises in our experiments, we would not expect our two stimulus paradigms to selectively activate opposite responses.

It might be argued further, however, that a local spatial gradient in endogenous Ca^{2+} buffering exists. For example, there might be more buffering in dendritic shafts than spines, resulting in a larger $[Ca^{2+}]_i$ elevation in spines than shafts, and such local $[Ca^{2+}]_i$ gradients might be essential for the reliable induction of LTP or LTD. However, any such differences in buffering would be present in all our experiments and would always lead to the same gradient in $[Ca^{2+}]_i$, for example, between spines and shafts. Despite such postulated gradients, we find that a prolonged modest photolyzing light reliably induces LTD, while a more intense brief photolysis only induces LTP. Thus the possible existence of such gradients cannot explain the differential responses we find to our two stimulus paradigms.

It is surprising that a brief modest rise in $[Ca^{2+}]_i$ can elicit either LTD or LTP, whereas a larger but still brief rise in $[Ca^{2+}]_i$ triggers LTP 36% of the time, but never LTD. The larger $[Ca^{2+}]_i$ increase appears to actively suppress LTD induction. Similarly, prolonging a modest rise in $[Ca^{2+}]_i$ appears to selectively inhibit LTP induction. We explored only a small subset of the two-dimensional space of magnitude and duration of $[Ca^{2+}]_i$ elevation. It would be interesting to map out this

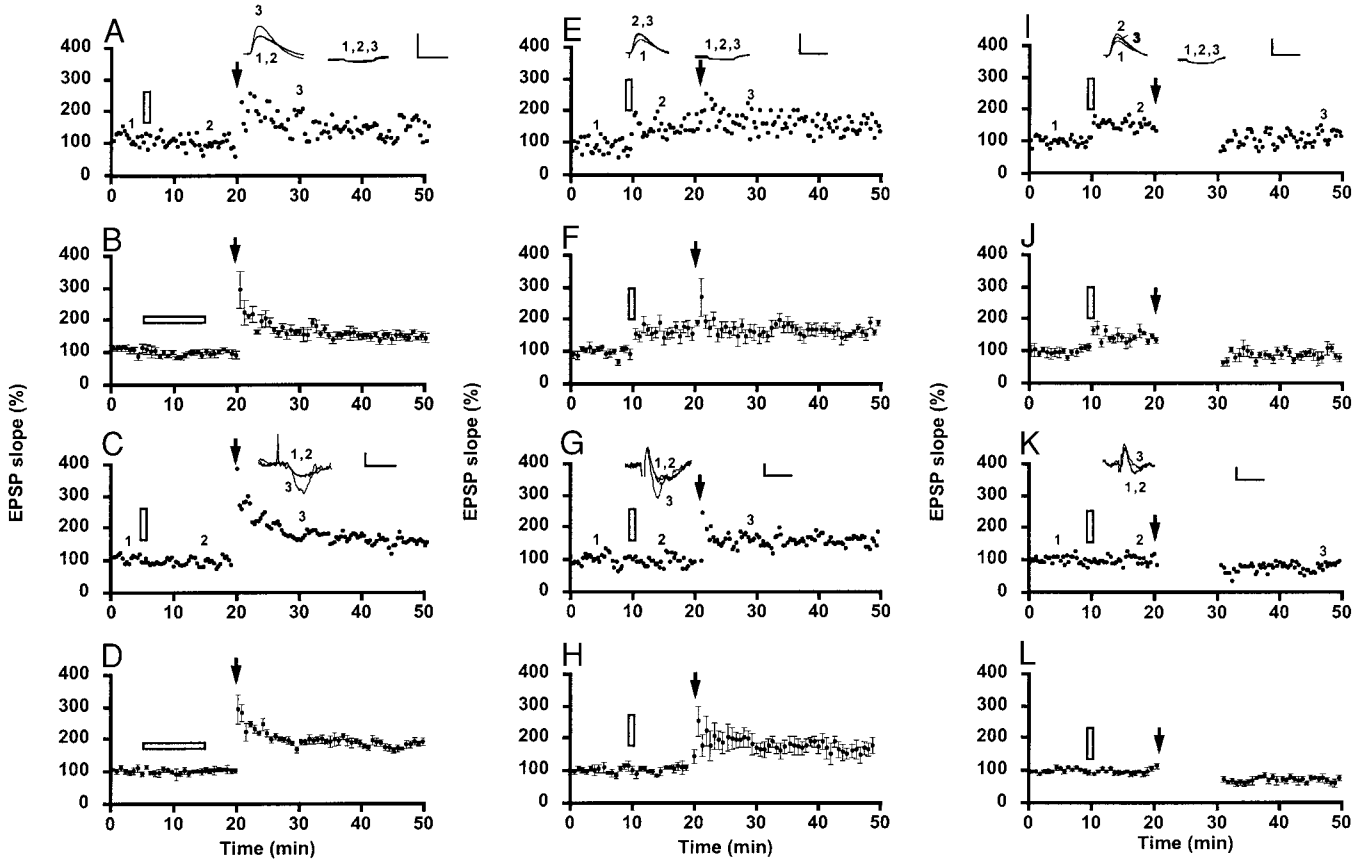

FIG. 4. Properties of slices exposed to LTP-inducing protocol. *Left panels* are from a slice in which photolysis (vertical bar) failed to induce LTP. This was followed by tetanic afferent stimulation (3 100-Hz trains for 1 s with 1 s pauses, at time marked by vertical arrow), which induced LTP. *Middle panels* are from a slice in which photolysis (vertical bars) induced LTP, which occluded induction of LTP to subsequent tetanus (vertical arrows). *Right panels* are from a slice in which photolysis-induced LTP (vertical bars) was followed by low-frequency afferent stimulation (1 Hz for 10 min, beginning at vertical arrow), which depotentiated the responses. *A*, *E*, and *I*: whole cell EPSP recordings from neurons filled with nitrophenyl-EGTA. *B*, *F*, and *J*: summary graphs of averaged EPSPs from 7 (*B*) or 6 (*F* and *J*) slices. Horizontal bar in *B* and *D* shows range of times when photolysis occurred in different experiments. *C*, *G*, and *K*: field potential recordings. *D*, *H*, and *L*: summary graphs of averaged field potential recordings from 7 (*D*) or 6 (*H* and *L*) slices. Insets and calibration bars as in Fig. 1.

space more extensively. What are the boundaries delimiting LTP and LTD induction? What effect would a prolonged intense rise in $[Ca^{2+}]_i$ have? Limitations of the current methodology prevent addressing the latter question.

Although we often observe LTP or LTD in response to a postsynaptic $[Ca^{2+}]_i$ elevation, we never induced the more transient short-term potentiation (STP) sometimes observed in response to briefer or less intense tetanic stimulation or to repeated postsynaptic depolarization (Colino et al. 1992; Huang et al. 1992; Kullmann et al. 1992; Malenka 1991; Malenka et al. 1992). Equally surprising is that in none of the studies on STD was LTD ever induced. This apparent discrepancy may be due to the ages of rats used. The STD studies were all done on slices from 2- to 8-wk-old rats, whereas our experiments were done on slices from 11- to 22-day-old rats. LTD is much more readily observed in the younger animals (Dudek and Bear 1993), where it may replace STD as the primary response to a modest rise in $[Ca^{2+}]_i$.

In some ways these results might have been anticipated from previous findings. For example, electrical induction of LTD of ~13% results from low-frequency afferent stimulation lasting for 30 s (Mulkey and Malenka 1992), and 1 min of stimulation produces depression of ~25%, similar to what we produced (25.8%) when $[Ca^{2+}]_i$ was elevated for ~1 min. LTP is elicited by tetanic stimulation raising $[Ca^{2+}]_i$ to significantly higher levels (Otani and Connor 1996; Petrozzino et al. 1995), but the $[Ca^{2+}]_i$ must remain elevated for a few seconds (Malenka et al. 1992). Our LTP-inducing protocol probably elevates $[Ca^{2+}]_i$ to sufficient levels but for just barely long enough to induce LTP. There seems to be a minimum rise in $[Ca^{2+}]_i$ that is essential for inducing LTP reliably, without inducing LTD. For example, pairing low-frequency afferent stimulation with postsynaptic depolarization results in LTP rather than LTD (Gustafsson et al. 1987), presumably because of the greater elevation in $[Ca^{2+}]_i$ resulting from unblocking NMDA receptors by depolarization. Repetitive afferent stimulation can elicit LTP or LTD, depending on the phase relationship between the stimulation and θ-burst oscillations (Huerta and Lisman 1995); at least part of this phase dependency is related to postsynaptic potential changes that can sensitize or suppress activation of Ca^{2+} influx through NMDA receptors. Moreover, a short postsynaptic depolarization alone can induce LTD (Cummings et al. 1996); presumably this treatment does not elevate postsynaptic $[Ca^{2+}]_i$ sufficiently to reliably induce LTP. Brief afferent tetanic stimulation that normally induces LTP can be made to induce LTD when postsynaptic depolarization is prevented, presumably because of reduction in Ca^{2+} influx (Cum-

mings et al. 1996). Thus it is clear that a brief rise in postsynaptic $[Ca^{2+}]_i$ can induce either LTP or LTD and that larger rises are more likely to induce LTP. In contrast, a prolonged modest $[Ca^{2+}]_i$ elevation selectively induces only LTD.

How might different amplitude and temporal patterns of postsynaptic $[Ca^{2+}]_i$ elevation selectively activate LTD and LTP? At least two classes of mechanism come to mind. One possibility is that the target molecules sensing $[Ca^{2+}]_i$ and responsible for inducing LTD and LTP, thought to be Ca-dependent phosphatases and kinases, respectively (Bliss and Collingridge 1993; Linden and Conner 1995), are preferentially activated by different patterns of $[Ca^{2+}]_i$ elevation. Such a $[Ca^{2+}]_i$ pattern sensitivity was reported in biochemical studies of calcium- and calmodulin-dependent kinase type II (De Koninck and Schulman 1998). Another possibility is that the phosphatase and kinase cascades that underlie LTD and LTP, respectively, may not only operate linearly to ultimately phosphorylate or dephosphorylate one or more final targets; rather the two cascades may interact and inhibit each other at one or more intermediate stages. Such a scheme of enzyme interactions resembles the multilayered and mutually inhibitory neural circuits involved in pattern recognition (Bishop 1995). The mechanisms of pattern recognition in "enzyme circuits" may be similar to those of neural circuits.

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REFERENCES

ARTOLA, A. AND SINGER, W. Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation. *Trends Neurosci.* 16: 480–487, 1993.

BISHOP, C. M. *Neural Networks for Pattern Recognition*. Oxford: Clarendon Press, 1995.

BLISS, T.V.P. AND COLLINGRIDGE, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31–39, 1993.

BORTOLOTTO, Z. A., BASHIR, Z. I., DAVIES, C. H., AND COLLINGRIDGE, G. L. A molecular switch activated by metabotropic glutamate receptors regulates induction of long-term potentiation. *Nature* 368: 740–743, 1994.

COHEN, A. S. AND ABRAHAM, W. C. Facilitation of long-term potentiation by prior activation of metabotropic glutamate receptors. *J. Neurophysiol.* 76: 953–962, 1996.

COLINO, A., HUANG, Y.-Y., AND MALENKA, R. C. Characterization of the integration time for the stabilization of long-term potentiation in area CA1 of the hippocampus. *J. Neurosci.* 12: 180–187, 1992.

CUMMINGS, J. A., MULKEY, R. M., NICOLL, R. A., AND MALENKA, R. C. Ca^{2+} signaling requirements for long-term depression in the hippocampus. *Neuron* 16: 825–833, 1996.

DE KONINCK, P. AND SCHULMAN, H. Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science* 279: 227–230, 1998.

DOLMETSCH, R. E., LEWIS, R. S., GOODNOW, D. D., AND HEALY, J. I. Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. *Nature* 386: 855–858, 1997.

DUDEK, S. M. AND BEAR, M. F. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J. Neurosci.* 13: 2910–2918, 1993.

ELLIS-DAVIES, G.C.R. AND KAPLAN, J. H. Nitrophenyl-EGTA, a photolabile chelator that selectively binds Ca^{2+} with high affinity and releases it rapidly upon photolysis. *Proc. Natl. Acad. Sci. USA* 91: 187–191, 1994.

ELLIS-DAVIES, G.C.R., KAPLAN, J. H., AND BARSOTTI, R. J. Laser photolysis of caged calcium: rates of calcium release by nitrophenyl-EGTA and DM-nitrophen. *Biophys. J.* 70: 1006–1016, 1996.

GABSO, M., NEHER, E., AND SPIRA, M. E. Low mobility of the Ca^{2+} buffers in axons of cultured Aplysia neurons. *Neuron* 18: 473–481, 1997.

GUSTAFSSON, B., WIGSTROM, H., ABRAHAM, W. C., AND HUANG, Y.-Y. Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *J. Neurosci.* 7: 774–780, 1987.

HELMCHEN, F., IMOTO, K., AND SAKMANN, B. Ca^{2+} buffering and action potential-evoked Ca^{2+} signaling in dendrites of pyramidal neurons. *Biophys. J.* 70: 1069–1081, 1996.

HUANG, Y.-Y., COLINO, A., SELIG, D. K., AND MALENKA, R. C. The influence of prior synaptic activity on the induction of long-term potentiation. *Science* 255: 730–733, 1992.

HUERTA, P. T. AND LISMAN, J. E. Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. *Neuron* 15: 1053–1063, 1995.

KULLMANN, D. M., PERKEL, D. J., MANABE, T., AND NICOLL, R. A. Ca^{2+} entry via postsynaptic voltage-sensitive Ca^{2+} channels can transiently potentiate excitatory synaptic transmission in the hippocampus. *Neuron* 9: 1175–1183, 1992.

LANDÒ, L. AND ZUCKER, R. S. "Caged calcium" in Aplysia pacemaker neurons. Characterization of calcium-activated potassium and nonspecific cation currents. *J. Gen. Physiol.* 93: 1017–1060, 1989.

LINDEN, D. J. AND CONNOR, J. A. Long-term synaptic depression. *Annu. Rev. Neurosci.* 18: 319–357, 1995.

LISMAN, J. A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proc. Natl. Acad. Sci. USA* 86: 9574–9578, 1989.

MALENKA, R. C. Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. *Neuron* 6: 53–60, 1991.

MALENKA, R. C., LANCASTER, B., AND ZUCKER, R. S. Temporal limits on the rise in postsynaptic calcium required for the induction of long-term potentiation. *Neuron* 9: 121–128, 1992.

MULKEY, R. M. AND MALENKA, R. C. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* 9: 967–975, 1992.

NEHER, E. AND ZUCKER, R. S. Multiple calcium-dependent processes related to secretion in bovine chromaffin cells. *Neuron* 10: 21–30, 1993.

NEVEU, D. AND ZUCKER, R. S. Postsynaptic levels of $[Ca^{2+}]_i$ needed to trigger LTD and LTP. *Neuron* 16: 619–629, 1996a.

NEVEU, D. AND ZUCKER, R. S. Long-lasting potentiation and depression without presynaptic activity. *J. Neurophysiol.* 75: 2157–2160, 1996b.

OTANI, S. AND CONNOR, J. A. Rapid dendritic Ca^{2+} influx is associated with induction of homosynaptic long-term depression in adult rat hippocampus. *Eur. J. Pharmacol.* 318: R5–R6, 1996.

PETROZZINO, J. J., POZZO MILLER, L. D., AND CONNOR, J. A. Micromolar Ca^{2+} transients in dendritic spines of hippocampal pyramidal neurons in brain slice. *Neuron* 14: 1223–1231, 1995.

PUSCH, M. AND NEHER, E. Rates of diffusional exchange between small cells and a measuring patch pipette. *Pflügers Arch.* 411: 204–211, 1988.

REGEHR, W. G. AND TANK, D. W. Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. *J. Neurosci.* 12: 4202–4223, 1992.

TSAI, K. Y., CARNEVALE, N. T., CLAIBORNE, B. J., AND BROWN, T. H. Efficient mapping from neuroanatomical to electrotonic space. *Network* 5: 21–46, 1994.

ZUCKER, R. S. Photorelease techniques for raising or lowering intracellular Ca^{2+}. *Methods Cell Biol.* 40: 31–63, 1994.