Bidirectional Synaptic Plasticity Correlated With the Magnitude of Dendritic Calcium Transients Above a Threshold

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Cormier, R. J., A. C. Greenwood, and J. A. Connor. Bidirectional synaptic plasticity correlated with the magnitude of dendritic calcium transients above a threshold. J Neurophysiol 85: 399–406, 2001. The magnitude of postsynaptic Ca\(^{2+}\) transients is thought to affect activity-dependent synaptic plasticity associated with learning and memory. Large Ca\(^{2+}\) transients have been implicated in the induction of long-term potentiation (LTP), while smaller Ca\(^{2+}\) transients have been associated with long-term depression (LTD). However, a direct relationship has not been demonstrated between Ca\(^{2+}\) measurements and direction of synaptic plasticity in the same cells, using one induction protocol. Here, we used glutamate iontophoresis to induce Ca\(^{2+}\) transients in hippocampal CA1 neurons injected with the Ca\(^{2+}\)-indicator fura-2. Test stimulation of one or two synaptic pathways before and after iontophoresis showed that the direction of synaptic plasticity correlated with glutamate-induced Ca\(^{2+}\) levels above a threshold, below which no plasticity occurred (≈180 nM). Relatively low Ca\(^{2+}\) levels (180–500 nM) typically led to LTD of synaptic transmission and higher levels (>500 nM) often led to LTP. Failure to show plasticity correlated with Ca\(^{2+}\) levels in two distinct ranges: <180 nM and >450–600 nM, while only LTD occurred between these ranges. Our data support a class of models in which failure of Ca\(^{2+}\) transients to affect transmission may arise either from insufficient Ca\(^{2+}\) to affect Ca\(^{2+}\)-sensitive proteins regulating synaptic strength through opposing activities or from higher Ca\(^{2+}\) levels that reset activities of such proteins without affecting the net balance of activities. Our estimates of the threshold Ca\(^{2+}\) level for LTD (≈180 nM) and for the transition from LTD to LTP (≈540 nM) may assist in constraining the molecular details of such models.

METHODS

We prepared coronal hemispheric brain slices (350- to 400-μm thick) from adult male Sprague-Dawley rats (Harlan; 5–10 wk old) by standard humane methods involving quick decapitation of anesthetized animals (Connor and Cormier 2000). The slices were then maintained at 28°C in artificial cerebrospinal fluid (ACSF; containing, in mM: 126 NaCl, 3 KCl, 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 1 MgCl\(_2\), 2 CaCl\(_2\), and 10 dextrose; gassed with 95% O\(_2\)-5% CO\(_2\)) until use. All chemicals were from Sigma.

For experiments, individual slices were placed in a submersion chamber and perfused at 1–2 ml/min with ACSF at 31 ± 0.5°C. Intracellular recordings were made from CA1 neurons ∼75 μm below the slice surface, using glass micropipettes with tips filled with 12 mM fura-2 and shanks filled with a solution of 3 M KCl and 1 M K-acetate. The electrode resistance was initially ∼200 MΩ with fura-2 present but dropped to ∼120 MΩ in ∼20 min before data collection. An Axoclamp 2A amplifier in bridge mode was used to record membrane potential. To evoke excitatory postsynaptic potentials (EPSPs), one or two monopolar stimulating electrodes were placed in stratum radiatum with the tip (20 μm diameter) ∼100 μm below the slice surface. Stimulating electrodes were placed in the proximal third of s. radiatum to activate afferents on proximal dendrites or in the distal third to activate afferents on distal dendrites. Pairs of EPSPs separated by 50 ms were evoked by constant-current pulses (50–100 μA) every 15 s. After stopping the afferent stimulation used to evoke baseline EPSPs, an iontophoresis pipette (1 M glutamate, pH 7.0, ∼10 MΩ) was positioned 20–50 μm from the primary apical dendrite of the fura-filled neuron. Glutamate was ejected by five iontophoretic pulses (duration: 10 s, amplitude: 4 μA, interval: 60 s), and the pipette was then withdrawn from the slice 5 min before afferent stimulation was then withdrawn from the slice.

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Relative to the start of the 10-s glutamate pulse. Vertical scale bar: 100 nM Ca²⁺.

**RESULTS**

We measured intracellular Ca²⁺ levels during iontophoresis of glutamate onto the apical dendrites of CA1 pyramidal cells in coronal brain slices from rats \((n = 67)\). The bulk of these experiments \((n = 53)\) served to characterize the ensuing Ca²⁺ transients, guiding a study of the relation between these transients and effects on transmission in 20 synaptic inputs to 14 cells with one \((n = 8)\) or two \((n = 6)\) stimulated synaptic pathways. Cells that were included in this study had stable resting potentials more negative than −65 mV and stable resting Ca²⁺ levels averaging 71 ± 11 (SE) nM.

Glutamate iontophoresis (10 s, 4 μA) typically depolarized the membrane to values between −50 and −10 mV (data described in this paragraph not shown) (see Cormier and Kelly 1996; Cormier et al. 1993). After a glutamate pulse, cells repolarized and sometimes also hyperpolarized, perhaps from activation of Ca²⁺-gated potassium channels (Hotson and Prince 1980). Action potentials were often observed during the first two glutamate pulses in a train (at 1 pulse/min) but rarely during later pulses, though these later pulses were associated with more rapid depolarization than were the first two pulses. Control experiments with equimolar substitution of sodium chloride for glutamate demonstrated that iontophoresis current (up to 10 times that used here) had no detectable effect on membrane potential, intracellular Ca²⁺, or synaptic transmission \((n = 3)\) (see Cormier and Kelly 1996; Cormier et al. 1993).

Focusing first on the temporal information in our imaging data, we measured Ca²⁺ levels in the region of dendrite to which glutamate was applied in most experiments, ~70 μm from the soma (marked in Fig. 1D, —). In this region, Ca²⁺ levels typically rose during 6–10 s of the standard 10-s pulse, reached a peak level between 100 and 600 nM, and returned to

![Fig. 1. Glutamate iontophoresis elicited reproducible Ca²⁺ transients.](https://jrn.physiology.org/...)

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**Fig. 1. Glutamate iontophoresis elicited reproducible Ca²⁺ transients.** A: time course of a representative transient elicited by a single 10-s pulse of glutamate \((4.2 \mu A)\). Glutamate was applied and Ca²⁺ was measured ~70 μm from the apical base. B: increasing the glutamate iontophoresis current increased the peak Ca²⁺ level in the same cell \(3.3, 4.2, \text{and} 5 \mu A\). Only 4-μA pulses were used to generate the Ca²⁺ transients that were related to plasticity in this study. C: a series of 5 glutamate pulses \(3.3, 4.2, \text{and} 5 \mu A\) had somewhat different effects in different cells. In 2 typical cells, successive Ca²⁺ transients were either larger \((C1)\) or similar \((C2)\). D: spatial profiles of Ca²⁺ transient induced by a single glutamate pulse and a fluorescent image of the fura-2 filled apical dendrite \((excited \ at 380 \ nm)\) from which the data came. The numbers to the left of the profiles indicate the measurement times relative to the start of the 10-s glutamate pulse. Vertical scale bar: 100 nM Ca²⁺. The location of the horizontal scale bar \(37 \ μm\) indicates the region closest to the glutamate pipet \(~70 \ μm\) from the apical base, coincident with the location of the peak Ca²⁺ levels \(—\). E: spatial profile of a Ca²⁺ transient peaking at the 8-s point during glutamate application to the distal dendrite \(~250 \ μm\) from the apical base, as indicated by location of the horizontal scale bar \(37 \ μm\) in the accompanying fura-2 picture. Not surprisingly, Ca²⁺ levels peaked more distally in the vicinity of the glutamate pipette, when glutamate was applied distally instead of to the proximal dendrite. The flat profile shows baseline Ca²⁺ levels. Vertical scale bar: 100 nM Ca²⁺.
basal levels by 5–120 s after the pulse (Fig. 1A: data from typical cell). Increasing the iontophoretic current produced higher Ca$^{2+}$ levels (Fig. 1B: data from same cell) and very prolonged Ca$^{2+}$ transients in some cases, as reported elsewhere (Connor and Cormier 2000). In our study relating Ca$^{2+}$ transients to plasticity, we set the iontophoretic current at an intermediate level (4 μA) that produced Ca$^{2+}$ levels <1 μM to reduce the risk of excitotoxic effects.

A series of five successive pulses of glutamate at one-minute intervals led to multiphasic Ca$^{2+}$ transients (Fig. 1C). In some experiments, the basal Ca$^{2+}$ level was higher for successive pulses ($n = 20$ of 67; typical data shown in Fig. 1C). Also the peak Ca$^{2+}$ level often increased with successive pulses ($n = 38$ of 67; Fig. 1C). Saturation of glutamate uptake (Asgtely et al. 1997; Mennerick and Zorumski 1995) or of Ca$^{2+}$ sequestration or extrusion, among other mechanisms, may have contributed to these phenomena. On the other hand, in many experiments, the peak Ca$^{2+}$ level was very similar from pulse to pulse ($n = 29$ of 67; Fig. 1C2 shows typical data).

The spatial distribution of glutamate-induced Ca$^{2+}$ transients within cells was inhomogeneous, suggesting that synapses at widely separated locations might show synaptic plasticity of differing magnitude and direction. In our initial experiments, we placed the iontophoresis pipet at ~70 μm along the proximal apical dendrites, producing Ca$^{2+}$ transients centered near the point of application. Typically, the initial peak was closer to the soma than the pipette was (Fig. 1D, 4 s), possibly the result of activating dendritic voltage-gated Ca$^{2+}$ channels (Miyakawa et al. 1992; Regehr and Tank 1992). However, by 8 s, the Ca$^{2+}$ levels most proximal to the soma had declined, leaving an increasingly well-defined peak centered near the glutamate source. In some later experiments ($n = 6$), the iontophoresis pipette was placed in distal stratum radiatum, ~250 μm from the base of the apical shaft. Distal glutamate led to Ca$^{2+}$ transients resembling those that followed proximal glutamate application, except that the location of maximal change tended to be shifted distally. An example is shown in Fig. 1E.

Having examined the effects of glutamate application on dendritic Ca$^{2+}$, and as a prelude to correlating glutamate-induced Ca$^{2+}$ levels with synaptic plasticity, we next asked what does glutamate iontophoresis do to synaptic transmission near and away from the application site in fura-filled cells? To answer this question, we used the fura-filled micropipette to record evoked intracellular EPSPs before and 15 min after glutamate application. In eight experiments, a single stimulating electrode was placed close to the cell-body layer to activate synapses at widely separated locations thus suited to answer the question: do the Ca$^{2+}$ levels that may have affected synaptic transmission in that dendritic region, prompting a series of two-pathway experiments with distal glutamate application.

In these two-pathway experiments ($n = 6$), the first stimulating electrode in proximal s. radiatum was complemented with a second one in distal s. radiatum, as diagrammed in Fig. 2A. Glutamate was applied to the dendrites in the distal third of s. radiatum (as in Fig. 1E) to increase the likelihood that EPSPs from the distal synapses would undergo LTP (as in the representative experimental time course in Fig. 2C). We expected that we might also observe plasticity in the proximal pathway due to the activation of voltage-gated Ca$^{2+}$ channels on the primary apical dendrites (Miyakawa et al. 1992; Regehr and Tank 1992). In fact, when we applied our plasticity-factor classification scheme to the proximal pathway, we observed no plasticity (98 ± 3%) in four cells, LTD (72%) in one cell, and LTP (302%) in one cell at 40–60 min after the glutamate treatment. Meanwhile, the distal pathway showed no plasticity (106 ± 1%) in two cells, LTD (32%) in one cell, and LTP (231 ± 51%) in three cells. These data are plotted on the right in Fig. 2B, with proximal and distal plasticity factors from individual neurons connected by lines. Thus both sides of Fig. 2B show that the range of observed plasticity factors was well suited to answer the question: do the Ca$^{2+}$ levels reached during glutamate iontophoresis and subsequent effects on synaptic transmission correlate as they would in a simple model in which Ca$^{2+}$ caused these effects?

To answer this question, we first pooled the electrophysiological data from the 8 single-pathway experiments and the six two-pathway experiments to obtain a total of 20 experimental time courses of EPSP slope, divided as before into three groups according to plasticity factor. The average time courses for the LTP, LTD, and no-plasticity groups of pooled data are plotted in Fig. 2B, D–F, respectively ($n = 7, 7,$ and 6). Next, we sorted the glutamate-induced Ca$^{2+}$ transients into the same three groups. In these data, the distal or proximal location of stimulus-activated synapses was inferred from the position of the stimulating electrode. To quantify the Ca$^{2+}$ levels that may have affected synaptic transmission, we analyzed the iontophoresis imaging data with measurement boxes placed on the dendrite near these putative synaptic locations. Figure 3A shows such box placement on a typical cell in a two-pathway experiment, while Fig. 3B shows the average multiple-pulse Ca$^{2+}$-level time course obtained from all such boxes for synaptic pathways that exhibited LTP. For comparison between pathways or groups of pathways, multiple-pulse time courses were collapsed to a single-pulse time course by averaging across the five glutamate pulses, as illustrated for the LTP

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1 In one cell, both pathways were unusually responsive to distal glutamate application. However, no other basis existed for excluding these data and their relation to corresponding Ca$^{2+}$ levels provided nonessential support for this study’s conclusions.
Then, the three points at 6, 8 and 10 s during the single-pulse time courses were averaged to obtain a single measure of peak-Ca$_{2+}$ level for each pathway or for each group in the case of the averaged data. Fura saturation had minimal effect on the average peak-Ca$_{2+}$ level, as at most one of five peaks in the average approached 1 mM (e.g., 850 nM in Fig. 1C).

Looking first at the results for each pathway separately, Fig. 3D shows a scatter plot of percentage plasticity factor versus the calculated peak Ca$_{2+}$ level. Different symbols indicate data collected from single (proximal) pathways and distal and proximal pathways in two-pathway experiments (see legend). Despite a statistically adequate fit ($r = 0.60, P < 0.005$), a linear model was rejected on physiological grounds because it predicted the ongoing induction of profound LTD at resting Ca$_{2+}$ levels, which of course was not observed. A linear model constrained to start from no plasticity at Ca$_{2+}$ baseline was rejected ($r = 0.12, P < 0.59$, not plotted). A fourth-order polynomial fit is also plotted in Fig. 3D to help visualize the multiphasic dependence of plasticity on Ca$_{2+}$ that a satisfactory model would need to explain. One satisfactory class of models would involve a balance of effectors with opposing effects on synaptic strength, with the activity of at least one effector being reset by Ca$_{2+}$ levels above a threshold (Grzywacz and Burgi 1998; Lisman 1989). In such a model, the no-plasticity group would be predicted to consist of two subgroups, one that was exposed to Ca$_{2+}$ levels below that required to reset the enzyme activities and a second that was exposed to Ca$_{2+}$ levels at which the activities of individual enzyme molecules were reset without affecting the balance of activities.

Turning next to the summary data shown in Fig. 3E, pairwise comparisons among all three plasticity groups showed that the peak Ca$_{2+}$ levels in the LTP group were significantly larger than in the LTD group ($P < 0.05, n = 7$ and 7). Thus our data support the conventional view that low Ca$_{2+}$ levels lead to LTD, while higher levels lead to LTP. In contrast, the mean peak Ca$_{2+}$ level of the no-plasticity group barely differed from that of the LTD group (not significant, $P > 0.9, n = 6$ and 7). Meanwhile, the standard deviation (SD = 220) of the peak Ca$_{2+}$ levels in the no-plasticity group was so large that its mean was not significantly different from that of the LTP group ($P > 0.2, n = 6$ and 7). However, as suggested by Fig. 3D, this
extreme variability within the no-plasticity group could be accounted for by dividing it into two subgroups (“low” and “high” Ca\(^{2+}\)) with markedly different average peak Ca\(^{2+}\) levels with small standard deviations \[147 \pm 20 \text{ and } 541 \pm 63 \text{ (SD) nM, } n = 3 \text{ and 3}\].

Of course, the significance of this difference in Ca\(^{2+}\) levels could not be tested because membership in the subgroups depended on Ca\(^{2+}\), violating the standard assumption of independence. However, the apparent bimodality of the distribution of peak Ca\(^{2+}\) levels within the no-plasticity group was supported by the failure of a normal model to fit this distribution \((P \ll 0.001, \chi^2 = 35)\). Also, despite the marked difference between the Ca\(^{2+}\) levels associated with the high-Ca\(^{2+}\) and low-Ca\(^{2+}\) no-plasticity subgroups, it followed from the no-plasticity group’s definition that the mean plasticity factors associated with these subgroups were statistically indistinguishable (Fig. 3F, \(P = 0.27, n = 3 \text{ and 3}\)). Finally, although the mean peak Ca\(^{2+}\) level associated with LTP was not much higher than that of the high-Ca\(^{2+}\) no-plasticity subgroup (Fig. 3E, no statement of significance possible), this result is not surprising because the iontophoretic current (4 nA) was selected to achieve Ca\(^{2+}\) levels below 1 \(\mu\)M, typically \(\sim 500 \text{ nM}\). Previous work showed that larger iontophoretic currents led to LTP (Cormier et al. 1993) and larger Ca\(^{2+}\) transients (Connor and Cormier 2000).

Returning to Fig. 3D, the fourth-order polynomial fit provided initial estimates for the Ca\(^{2+}\) levels associated with the low-Ca\(^{2+}\) transition between no plasticity and LTD (165 nM) and the 100% cross-over point between the LTD and LTP groups (485 nM). To address the arbitrariness of the fourth-order polynomial, we also estimated each of these transition points by a second method. For a second estimate of the low-Ca\(^{2+}\) transition, linear interpolation between the data points straddling the 85% plasticity line yielded an 85% inter-
cept of 180 nM. For a second estimate of the 100% cross-over point, we took the mean peak Ca\(^{2+}\) level in the high-Ca\(^{2+}\) no-plasticity subgroup (541 nM). As they include no arbitrary assumptions, we accepted these second estimates in place of the estimates from the fourth-order polynomial, with which they are in reasonable agreement.

**DISCUSSION**

This paper reports that glutamate iontophoresis elevated intracellular Ca\(^{2+}\) in the apical dendrites of CA1 pyramidal cells and induced synaptic plasticity in the same cells. Although glutamate iontophoresis was previously shown to induce synaptic plasticity in hippocampal cultures (Malgaroli and Tsien 1992) and slices (Cormier and Kelly 1996; Cormier et al. 1993), the present study included Ca\(^{2+}\) imaging, allowing us to relate glutamate-induced Ca\(^{2+}\) levels to synaptic plasticity quantitatively.

**Glutamate iontophoresis, Ca\(^{2+}\), and cell viability**

The iontophoretic protocol produced Ca\(^{2+}\) transients that returned to baseline within 120 s after a series of five 10-s pulses at 1-min intervals. Earlier work suggested that exposure to appropriate Ca\(^{2+}\) levels for 5 min was sufficient to induce LTD (Mulkey and Malenka 1992), while only 2.5 s of appropriate Ca\(^{2+}\) levels was reported to be required for LTP induction (Malenka et al. 1992). With these putative temporal requirements in mind, our protocol was designed to reduce the importance of Ca\(^{2+}\) -transient kinetics as a factor affecting synaptic plasticity and to result in LTD or LTP, depending on the peak Ca\(^{2+}\) levels achieved. The between-cell variability that we observed in peak Ca\(^{2+}\) levels is consistent with the many sources of variability inherent in the iontophoretic application of glutamate in the slice, including cell depth, variations in local perfusate flow, and distance from the dendrite. This variability is also consistent with an earlier study of glutamate-induced Ca\(^{2+}\) transients in dentate granule cells, not involving synaptic plasticity (Kudo et al. 1987).

In contrast to our protocol, which required five sustaining 10-s pulses of glutamate to achieve \(\sim 5\) min of elevated Ca\(^{2+}\), a neurotoxic exposure of acutely dissociated hippocampal cells to glutamate or N-methyl-D-aspartate (NMDA) can induce Ca\(^{2+}\) transients that last for many minutes after iontophoresis (Connor et al. 1988; Wadman and Connor 1992). Although we found that a similarly prolonged recovery could be achieved using glutamate iontophoresis in hippocampal slices, in our hands it required twice as much iontophoresis current as was used in the present study (Connor and Cormier 2000). Thus our five-pulse protocol was designed to achieve relatively prolonged Ca\(^{2+}\) transients, while avoiding the prolonged single-pulse recovery that we feared would be associated with neurotoxic effects. As the higher Ca\(^{2+}\) levels were associated with LTP, whereas sick cells might be expected to show LTD, we infer that we successfully avoided complicating neurotoxic effects. This inference is further supported by our observation of stable resting potential and input resistance throughout the experiments.

**Glutamate iontophoresis and synaptic plasticity**

The clear correlation between Ca\(^{2+}\) levels and plasticity that we observed suggests that Ca\(^{2+}\) is a key mediator of plasticity induced by glutamate iontophoresis. However, our experiments do not rule out effects of glutamate on synaptic plasticity that are not entirely mediated by postsynaptic Ca\(^{2+}\). For example, in addition to their role in mobilizing intracellular Ca\(^{2+}\) (Jaffe and Brown 1994; Linden et al. 1994; Llano et al. 1991; Murphy and Miller 1988), metabotropic glutamate receptors can affect synaptic transmission in a variety of other ways (Conn and Pin 1997). Also, glutamate iontophoresis may release neuromodulators, such as nitric oxide or arachidonic acid (Medina and Izquierdo 1995). Addressing the possibility that these substances or glutamate contributed to presynaptic plasticity in our experiments, we note that we did not stimulate afferent fibers during glutamate exposure and that previous work established that presynaptic action potentials and transmitter release are not required for the induction of plasticity by glutamate iontophoresis (Cormier et al. 1993).

In contrast to the absence of synaptic stimulation in our induction protocol, an earlier study found that sustained dendritic Ca\(^{2+}\) transients elicited by action potentials evoked with current pulses at 3 Hz for 5 min were insufficient to elicit LTD without paired synaptic stimulation (Christie et al. 1996). This study also differed from the present study in that \(\Delta F/F\) measurements of fura-2 fluorescence were reported instead of ratiometrically determined Ca\(^{2+}\) levels, preventing a direct comparison of results. However, in harmony with the present study, these authors interpreted their results to suggest that Ca\(^{2+}\) played a critical role in the efficacy of their LTD-induction protocol, noting that LTD induction was blocked by nimodipine, Ni\(^{2+}\), or APV (independently) and suggesting that the essential role of paired synaptic stimulation may have been to enhance Ca\(^{2+}\) influx specifically into spines (see also Yuste and Denk 1995). These localized synaptic Ca\(^{2+}\) transients apparently did not add significantly to the dendritic \(\Delta F/F\) observed with action potentials alone. In contrast, our admittedly less “physiological” induction protocol may have permitted a more direct assessment of the Ca\(^{2+}\) levels affecting transmission, as glutamate-induced Ca\(^{2+}\) transients are relatively homogeneous spatially, especially in fura-filled cells (see following section).

**Ca\(^{2+}\) measurements with fura-2**

Two concerns naturally arise regarding the use of fura-2. First, its high affinity for Ca\(^{2+}\) (\(\sim 225\) nM) could lead to saturation. However, saturation occurs at \(\sim 1\) \(\mu\)M Ca\(^{2+}\), above the levels that we obtained during plasticity induction. Second, it has been reported that exogenous Ca\(^{2+}\) buffers, like fura-2, can interfere with the induction of LTP by diminishing the peak amplitude of brief Ca\(^{2+}\) transients while incidentally prolonging the recovery (Hansel et al. 1996, 1997; Kimura et al. 1990; Lynch et al. 1983; Malenka et al. 1988). However, the use of fura-2 in our experiments is unlikely to have interfered with the relation between our Ca\(^{2+}\) measurements and plasticity for the following reasons. Similar methods were reported to lead to intracellular fura levels of 20–30 \(\mu\)M (Petrozzino et al. 1995), while our conservatively high calculated estimate was 60 \(\mu\)M. Also, as the postsynaptic Ca\(^{2+}\) transients were induced by iontophoresis of glutamate at some distance from the putative synaptic location, the transients were expected to be inherently slow, reducing concerns that fura-2 would further retard their kinetics or diminish their magnitude. Granted, it is
likely that mobile fura-2 molecules tended to keep the Ca\textsuperscript{2+} level at the synapse and in the dendrite more homogeneous than if local Ca\textsuperscript{2+} level depended only on local Ca\textsuperscript{2+} channels and other physiological sources (Carnevale and Rosenthal 1992). However, this enhanced homogeneity coupled with our slow Ca\textsuperscript{2+}-transient kinetics would have the advantage of reducing extra variance in the relationship between measurements of plasticity and dendritic Ca\textsuperscript{2+}. In summary, the main goal of these experiments was to study effects of Ca\textsuperscript{2+}-transient magnitude in relative isolation from duration effects, irrespective of “normal” Ca\textsuperscript{2+}-level kinetics and spatial homogeneity.

Synapse localization

Nonetheless, imprecision in synapse localization must have contributed to variance in the relationship between the Ca\textsuperscript{2+} measurements and synaptic plasticity, tending to obscure our results. To limit this source of variance, we placed a fine stimulating electrode (20 μm diameter tip) as close as 0.5 mm from the dendrite and measured “plasticity-related” Ca\textsuperscript{2+} along a ~40 μm length of dendrite. Also relevant to the probable magnitude of this source of variance, the Ca\textsuperscript{2+} gradient along the apical dendrite was typically ~1 nM/μm within 50 μm of the presumed synaptic locations at the time of peak Ca\textsuperscript{2+}. In spite of this variance, however, some significant and intriguing results emerged from the data.

Distinct Ca\textsuperscript{2+} thresholds for LTD and LTP

These data provided indirect support for a class of models in which Ca\textsuperscript{2+} levels above a threshold reset the activity-level balance between opposing molecular effectors of synaptic strength (e.g., Lisman 1989). Specifically, we observed LTD at lower peak Ca\textsuperscript{2+} levels and LTP at higher levels (means: 335 ± 46 and 574 ± 27 nM, P < 0.05). Our data also provide estimates of the transition Ca\textsuperscript{2+} levels that separate the LTD plasticity group from the no-plasticity subgroup on the low-Ca\textsuperscript{2+} side (180 nM) and from the LTP group on the high-Ca\textsuperscript{2+} side (541 nM). The latter transition level is the mean peak Ca\textsuperscript{2+} level of the no-plasticity subgroup intermediate between LTD and LTP, with peak Ca\textsuperscript{2+} levels ranging from 450 to 600 nM. Ours is the first study to document the existence of this no-plasticity subgroup, an essential prediction of a Ca\textsuperscript{2+}-based version of the BCM learning rule (Bienenstock et al. 1982).

In this study, we focused on peak Ca\textsuperscript{2+} levels because prior evidence suggested that peak Ca\textsuperscript{2+} levels were related to synaptic plasticity (Hansel et al. 1996, 1997; Malenka et al. 1988; Müller and Connor 1991; Neveu and Zucker 1996; Petrozzino et al. 1995; Yuste and Denk 1995). Of course, it is likely that Ca\textsuperscript{2+} levels during the decay phase also affect synaptic plasticity. However, because decay is fast when Ca\textsuperscript{2+} is high, most of the recovery phase (the long tail) is similar for a transient peaking at 600 nM and one peaking at 300 nM, for example. To a rough approximation, the main difference between such transients is the peak at 600 nM and swift decay to 300 nM. This difference in peak Ca\textsuperscript{2+} may contribute a difference in net plasticity, while the remaining decay phase in common may make similar contributions to net plasticity. Our use of five relatively closely spaced transients, interrupting the first four decay phases, may accentuate the effects on plasticity of differences in peak Ca\textsuperscript{2+}.

Relating our findings to earlier work, this laboratory previously showed that increasing Ca\textsuperscript{2+} levels to 20 μM by tetanic stimulation (Petrozzino et al. 1995) or bath application of tetraethylammonium (Petrozzino and Connor 1994) reliably induced robust LTP. These early studies differ from the present study in that a low-affinity indicator (mag-fura-5) was used to focus exclusively on LTD induced by large Ca\textsuperscript{2+} transients in spines and fine dendrites. Also an early study where Ca\textsuperscript{2+} transients were produced by flash-photolysis of the caged-Ca\textsuperscript{2+} compound nitr-5 that was injected into the postsynaptic cell showed that 2–4 μM Ca\textsuperscript{2+} induced only LTP (Malenka et al. 1988). A more recent nitr-5 study suggested that Ca\textsuperscript{2+} levels of 300–500 nM induced LTD and LTP in separate cells (Neveu and Zucker 1996). Unlike the present study, this study did not measure the Ca\textsuperscript{2+} levels resulting from the plasticity-induction protocol (flash photolysis) and, instead, estimated Ca\textsuperscript{2+} from the duration of the flash, cell depth, and model assumptions. Further support for distinct Ca\textsuperscript{2+} thresholds came from imaging studies where different stimulation protocols induced LTD or LTP and caused relatively small and large Ca\textsuperscript{2+} transients, respectively (Hansel et al. 1996, 1997). However, the synaptic plasticity and imaging measurements in these studies were conducted in separate experiments and calibrated estimates of Ca\textsuperscript{2+} levels were not provided (Hansel et al. 1996, 1997).

Another study used two tetanic protocols in the presence or absence of picrotoxin to evoke Ca\textsuperscript{2+} transients that were related to LTD and LTP (Otani and Connor 1998). Several factors motivated the execution of the present study with a single protocol of glutamate iontophoresis, achieving results that extend the results of these earlier experiments without contradiction. Compared to glutamate iontophoresis, tetani are likely to activate different mechanisms of Ca\textsuperscript{2+} entry and additional plasticity factors. As a result of recruitment of additional pathways, the spatial domain of the Ca\textsuperscript{2+} transients associated with tetani may not accurately reflect the synaptic locations under test conditions. Also, Ca\textsuperscript{2+} flows directly into the postsynaptic structure during tetani, reaching transient local peaks that are likely to affect plasticity but are difficult to measure accurately. In contrast, glutamate iontophoresis generated slow and relatively homogeneous Ca\textsuperscript{2+} increases that could be measured accurately. Despite these methodological differences, however, no contradiction exists between the results of these two studies. The mean peak Ca\textsuperscript{2+} associated with LTD in the earlier tetanic study was ~460 nM, consistent with the range of peak levels determined for LTD in the present study (180–500 nM). The tetanic value could be a little high within the present range because Ca\textsuperscript{2+} levels were near their peak for only ~10 s with the tetanic protocol or because baseline Ca\textsuperscript{2+} was relatively high in the earlier study. Conditions of insufficient Ca\textsuperscript{2+} increase for plasticity were also consistent between studies. In the earlier study, no plasticity was observed under tetanic conditions leading to a peak Ca\textsuperscript{2+} increase of ~30 nM from baseline, safely below our new threshold for the induction of LTD (an increase of 120 nM from baseline). Finally, the mean Ca\textsuperscript{2+} increase associated with LTD in the earlier tetanic study was >1 μM, well above our minimum threshold for LTD induction.

In summary, our new results are consistent with earlier data while going beyond them to show, with one induction protocol and one Ca\textsuperscript{2+} indicator, that low Ca\textsuperscript{2+} levels induce LTD, high Ca\textsuperscript{2+} levels induce LTP, and intermediate Ca\textsuperscript{2+} levels are
associated with a no-plasticity domain. In general, of course, Ca\(^{2+}\) thresholds for the induction of synaptic plasticity are likely to be affected by Ca\(^{2+}\)-signal duration and various pre- and postsynaptic factors, perhaps including prior neuronal activity (Abraham and Tate 1997; Bienenstock et al. 1982). During our iontophoretic protocol, however, prolonged postsynaptic Ca\(^{2+}\) transients are uncoupled from presynaptic activity. With this protocol, it may be that a steady-state balance of Ca\(^{2+}\)-dependent enzymes plays a relatively large role in determining the ensuing synaptic plasticity. Thus our estimates of the threshold Ca\(^{2+}\) level for LTD (~180 nM) and for the transition from LTD to LTP (~540 nM) in this simplified context may contribute to the ongoing effort to construct a molecular model of Ca\(^{2+}\)-dependent synaptic plasticity (Grzywacz and Burgi 1998; Lisman 1989; Malenka and Nicoll 1999; Soderling and Derkach 2000).

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