Local Dendritic Ca^{2+} Signaling Induces Cerebellar Long-Term Depression

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Abstract

The coordinated activity of large numbers of adjacent parallel fiber synapses elevate calcium concentration locally in small regions of Purkinje cell dendrites. Such activity has also been reported to produce long-term depression of parallel fiber synaptic transmission. We have examined the relationship between these two events by combining patch clamp measurements of parallel fiber synaptic transmission with confocal microscopic imaging of the local calcium signals. We find that patterns of parallel fiber activity capable of evoking long-term depression invariably cause increases in Purkinje cell calcium concentration that are very spatially restricted. These results suggest that one function of the local dendritic calcium signals is to induce long-term depression of parallel fiber synapses.

Introduction

Long-term depression (LTD) is a form of plasticity expressed by a number of different synapses (for review, see Linden 1994). LTD was first documented in the cerebellar cortex. Here, the seminal observations of Ito and colleagues (Ito and Kano 1982; Ito et al. 1982) established that LTD results

from the coincident activation of parallel fiber (PF) and climbing fiber (CF) inputs onto Purkinje cells. LTD causes a long-lasting decrease in the efficacy of PF synaptic transmission without a comparable change in CF transmission. Given the importance of the cerebellum for motor learning (Ito 1984; Krupa et al. 1993; Aiba et al. 1994), it has been widely proposed that LTD may serve as a cellular substrate of motor learning and memory in cerebellar cortical circuits (Ito 1989).

The associative nature of LTD requires a coincidence detector that senses signals produced by both PF and CF activity (Bourne and Nicoll 1993). Although our understanding of this coincidence detector is still at an early stage (Ito 1991; Linden 1994; Konnerth et al. 1996), there has been substantial progress in determining the signaling events involved in LTD. Three lines of evidence have established that a CF-induced rise in Ca^{2+} concentration within the postsynaptic Purkinje cell serves as an initiating signal for LTD. First, activation of CF synapses is associated with a transient rise in intracellular Ca^{2+} concentration (Ross and Werman 1987; Konnerth et al. 1992). This increase in Ca^{2+} concentration occurs throughout much (or all) of the dendritic field of the Purkinje cell. Second, a CF-induced rise in Ca^{2+} concentration is necessary for LTD because induction of LTD is prevented by injection of Ca^{2+} chelators into Purkinje cells (Sakurai 1990; Konnerth et al. 1992). Finally, an increase in postsynaptic Ca^{2+} concentration is sufficient to produce LTD because direct elevation of Ca^{2+} concentration—either by depolarization-induced activation of voltage-gated Ca^{2+} channels (Crepel and Jaillard 1991; Linden et al. 1991; Kon-

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nerth et al. 1992) or by photolysis of “caged Ca^{2+} ” compounds (Kasono and Hirano 1994; Lev-Ram et al. 1995)—can substitute for activation of the CF synapse. Thus, it is clear that CF activity initiates LTD by elevating postsynaptic Ca^{2+} concentration.

The nature of the signals resulting from PF activity is less clear. Various reports have established the importance of postsynaptic G-protein-coupled receptors, specifically the type I metabotropic glutamate receptors (Aiba et al. 1994; Hartell 1994). These receptors produce diacylglycerol and inositol 1,4,5-trisphosphate (IP_3), which also have been implicated in LTD (Crepel and Krupa 1988; Linden and Connor 1991, Kasono and Hirano 1995). Roles for postsynaptic cGMP (Hartell 1996b; V. Lev-Ram, in prep.), as well as presynaptic nitric oxide (Lev-Ram et al. 1995), have also been proposed. Under some conditions, PF stimulation also can elevate postsynaptic Ca^{2+} concentration (Miyakawa et al. 1992; Denk et al. 1995; Eilers et al. 1995a). Until recently, there has been little support for the idea (DeSchutter 1995; DeSchutter and Maex 1996) that these PF-induced Ca^{2+} signals can induce LTD. However a recent report indicates that strong activation of PFs induces LTD even in the absence of CF activity (Hartell 1996a). This PF-mediated LTD (PF-LTD) is occluded by the conventional LTD resulting from coactivation of CFs and PFs, suggesting that the signal produced by local recruitment of PFs can compensate for the activity of the CF. Like CF-induced LTD, PF-LTD depends on a rise in postsynaptic Ca^{2+} concentration. Thus, although an increase in postsynaptic Ca^{2+} concentration normally is the signaling contribution of the CF, under some conditions PF can also raise postsynaptic Ca^{2+} concentration sufficiently to produce LTD.

PF activity produces postsynaptic Ca^{2+} signals that vary widely in their spatial range. Single-shock PF stimulation can produce Ca^{2+} signals in terminal dendrites and their adjacent spines (Eilers et al. 1995a; Hartell 1996a), whereas repetitive PF stimulation can induce Ca^{2+} signals of variable area, ranging from a single spine (Denk et al. 1995) to large portions of the dendritic tree (Miyakawa et al. 1992). It is not yet clear which of these types of PF-induced Ca^{2+} signal are capable of producing LTD. Here, we address this issue by measuring postsynaptic Ca^{2+} concentration while inducing PF-LTD by subthreshold PF activity. We find that the Ca^{2+} transients responsible for PF-LTD induction are restricted to the terminal dendrites and immediate parent dendrites of Purkinje cells. This defines a functional role for these local dendritic

signals and demonstrates that induction of PF-LTD is a spatially restricted phenomenon that relies on the local electrical and biochemical properties of individual dendrites.

Materials and Methods

PREPARATION OF CEREBELLAR SLICES

Experiments were performed on 200- μm -thick cerebellar slices from 16- to 22-day-old Wistar rats. After decapitation of the animals, the cerebellum was removed rapidly and placed in ice-cold saline composed of 125 mM NaCl, 2.5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 1.25 mM NaH_2PO_4 , 26 mM NaHCO_3 , and 20 mM glucose and then bubbled with 95% O_2 /5% CO_2 (pH 7.3). Parasagittal slices were cut with a vibratome (Vibracut, FTB, Bensheim, Germany) and kept in saline at 35°C for 30–40 min. After this incubation period the slices were stored for up to 6 hr in saline at 25°C until they were used for experiments.

ELECTROPHYSIOLOGICAL RECORDINGS

Tight-seal whole-cell recordings were obtained from the somata of individual visually identified Purkinje cells (Edwards et al. 1989). Slices were transferred to a submerged recording chamber and perfused continuously with saline (20°C–22°C) to which 10 μM bicuculline methiodide was added. Patch pipettes (2–3 M Ω resistance) were pulled from borosilicate glass (Hilgenberg, Malsfeld, Germany) and filled with 140 mM KCl, 10 mM NaCl, 10 mM HEPES, 4 mM Mg-ATP, 0.4 mM Na-GTP, and 0.5 mM EGTA (pH 7.3 KOH). In some recordings 130 mM KCl was replaced by 130 mM potassium gluconate. For imaging experiments, EGTA was replaced by either the fluorescent calcium indicator dye Calcium Green 1 or Oregon BAPTA Green 1 (Molecular Probes, Eugene, OR). Voltage-clamp and current-clamp recordings were done with an EPC9 patch-clamp amplifier (HEKA, Lambrecht, Germany). For focal activation of PFs, an extracellular stimulation pipette was placed in the molecular layer (Konnerth et al. 1990; Llano et al. 1991a).

After breaking into the cell, PFs were stimulated (100 μs , 5–15 V) at a frequency of 0.1 or 0.2 Hz, and excitatory postsynaptic currents (EPSCs) were recorded in the voltage-clamp mode. When a stable baseline recording was obtained for at least 10 min, LTD-inducing stimuli were applied, and recordings were continued for another 30–120

min afterward. LTD was induced by one of two protocols: (1) Stimulus intensity was raised and the amplifier was set to current-clamp mode; in this case, PF-excitatory postsynaptic potentials (EPSPs) were evoked at 1 Hz for 1–2 min; or (2) the Purkinje cell was voltage clamped and stimulus intensity was kept constant while PFs were activated in brief bursts of stimuli (two to five pulses per train, at a frequency of 10–50 Hz) applied every second for 1–2 min. Peak amplitudes of the electrical responses were measured with Pulse (HEKA) and transferred to Igor Pro (Wavemetrics, Lake Oswego, OR) for further analysis. LTD was defined as an irreversible decrease of >20% in PF response amplitude.

FLUOROMETRIC CALCIUM IMAGING

Changes in the intracellular calcium concentration were recorded with a video-rate confocal laser-scanning system (Odyssey, Noran, Middletown, WI) that was mounted on an upright microscope (Axioplan, Zeiss; Eilers et al. 1995b) equipped with water-immersion objectives (Achromplan, Zeiss, 40 $\times$, NA 0.75 and 63 $\times$, NA 0.9). Full-frame images usually were collected at video rate (30 Hz) and stored on an optical disc recorder (TQ-2026F, Panasonic, Secaucus, NJ). Images were processed off-line with Image-1 software (Universal Image Co., West Chester, PA). In some experiments images were digitized on-line (MVP-AT frame-grabber board, Matrox, Dorval, Quebec, Canada), integrated over periods of 500 msec (Image 1) and stored on the hard disk of a personal computer. In either case, fluorescence data were reduced to graphic form by measuring fluorescence from areas of interest and calculating the background-corrected increase in fluorescence divided by the prestimulus fluorescence ($\Delta F/F$) using Igor Pro. Such ratios yield a parameter that is independent of optical path length and dye concentration and, at a given resting Ca^{2+} level, is a unique function of intracellular Ca^{2+} concentration (e.g., Neher and Augustine 1992). The laser was shuttered between recording episodes to minimize phototoxic damage. Experiments showing any signs of such damage (e.g., irreversible changes in fluorescence or increases in Purkinje cell holding current) were not accepted for analysis.

Results

We began our study of PF-LTD by defining experimental conditions that allowed us to reliably

evoke PF-LTD and then used imaging techniques to visualize the Ca^{2+} signaling events associated with this form of synaptic plasticity.

REQUIREMENTS FOR INDUCTION OF LTD BY PARALLEL FIBER ACTIVITY

Stimulation of PFs at relatively low intensity and low frequency (0.1–0.2 Hz) produced postsynaptic electrical responses that were stable over prolonged periods of time. These conditions were therefore chosen to monitor basal PF synaptic transmission. At low stimulation intensities, increasing the stimulation frequency to 1 Hz for 1 to 2 min did not consistently depress synaptic transmission ($n = 5$; data not shown; see also Hartell 1996a). However, more vigorous stimulation of PFs was capable of eliciting LTD. We established two PF stimulation protocols that reliably produced LTD. With both stimulus paradigms, PF transmission was partially depressed immediately after the stimulus. The magnitude of the depression then increased progressively over the next 30 min and was stable for the duration of the recording (up to 2 hr). In both cases, LTD caused a reduction in the amplitude of PF-EPSCs, without any change in their time course. It is important to note that neither of these PF stimulation paradigms evoked action potentials, as monitored by recordings from the Purkinje cell body.

In the first stimulus regimen, stimulation intensity was kept constant but PFs were activated with short bursts of stimuli (two to five pulses at 10–50 Hz) at a frequency of 1 Hz for 1 to 2 min. This protocol produced a depression of PF synaptic responses in 20 out of 29 cells, with a mean decrease in EPSC amplitude to $58\% \pm 2\%$ ($\pm$ S.E.M.) measured 25 min after the start of the induction (Fig. 1A). In the second paradigm, LTD was induced while switching the patch clamp amplifier from voltage clamp to current clamp mode. Turning off the voltage clamp allows the Purkinje cell membrane potential to depolarize more during a PF response, thereby increasing the amount of Ca^{2+} entry evoked by a given number of active PFs (Eilers et al. 1995a). In addition, stimulus intensity was increased to recruit additional PFs. Under these conditions, activation of PFs at 1 Hz for 1–2 min induced a long-lasting depression of PF responses in 8 out of 10 cells, with a mean decrease to $56\% \pm 1\%$ in the amplitude of PF-EPSCs (Fig. 1B).

Figure 1: Induction protocols for parallel fiber mediated LTD. Two induction protocols were used, both of which produced LTD that developed over a similar time course. (A) The first protocol consisted of short bursts of PF stimulation (two to five stimuli at 10–50 Hz), which were repeated at 1 Hz for 1–2 min. This stimulation protocol was given in voltage-clamp mode. For each experiment the responses were binned to 1 min, and the mean and S.E.M. values were calculated within the two experimental groups. Horizontal bars indicate the induction periods. Experiments were counted as failures when the reduction in synaptic response was <20%. EPSCs taken from a representative experiment are shown (*upper right*). The EPSCs were averaged over 2 min at the indicated time points. Scale bar, 100 pA/100 msec for EPSCs 1 and 3 and 100 pA/200 msec for EPSC 2. (B) The second protocol utilized single-shock stimulations of increased intensity, which were applied in current-clamp mode. Stimulation was repeated at 1 Hz for 1–2 min. Electrical responses taken from a representative experiment are shown (*upper right*). The responses were averaged over 2 min at the indicated time points. Scale bar, 20 pA/100 msec for response 1 and 3 and 2 mV/200 msec for response 2.

IMAGING PURKINJE CELL CALCIUM SIGNALS DURING INDUCTION OF LTD

The PF stimulation patterns that we have found to produce LTD, that is, burst stimulation and/or strong PF stimulation, are known to pro-

duce local and transient elevations in dendritic $[Ca^{2+}]_i$ (Miyakawa et al. 1992; Eilers et al. 1995a; Hartell 1996a). However, previous studies have left unresolved the questions of whether these dendritic Ca^{2+} signals remain spatially restricted during repetitive activity of PFs and whether local Ca^{2+} signals are indeed sufficient to produce LTD. We have addressed these questions by measuring postsynaptic Ca^{2+} concentration changes during the induction of PF-LTD.

To define the spatial range of postsynaptic Ca^{2+} signaling during PF-LTD induction, we combined whole-cell patch clamp recordings of Purkinje cell electrical responses with simultaneous imaging of changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The following data were obtained from 10 experiments in which simultaneous patch-clamp and imaging recordings were acquired from single Purkinje cells. To measure $[Ca^{2+}]_i$, Purkinje cells were filled with a Ca^{2+} indicator dye, such as Oregon BAPTA Green-1, which was delivered by the patch pipette. An image of a Purkinje cell filled with a fluorescent Ca^{2+} indicator dye is shown in Figure 2. This method of delivery causes a gradual increase in dye concentration as the dye diffuses from the patch pipette throughout the dendrites (Pusch and Neher 1988; Rexhausen 1992). We began our measurements at least 20 min after starting to introduce the dye; by this time the dye concentration should have been well equilibrated in the dendritic compartments under study and has been estimated to reach 40%–45% of the concentration

Figure 2: Confocal image of a cerebellar Purkinje cell from an 18-day-old rat. The cell was filled with the calcium indicator dye Calcium Green 1 via the somatic patch pipette (*right*). Afferent PFs were activated with an extracellular stimulation electrode (not shown) that was located in the molecular layer.

of dye within the pipette (Rexhausen 1992). Dye concentration should have increased no more than 5% during the course of LTD induction, so that any images acquired during this time are unlikely to be distorted significantly by time-dependent changes in dye concentration.

IDENTIFICATION OF THE SITE OF ACTIVE PF SYNAPSES

The dendritic tree of the Purkinje cell receives glutamatergic excitatory synaptic inputs from many thousands of PFs. Thus, one of the technical challenges in performing optical imaging of post-synaptic Ca^{2+} signaling during PF-LTD is to determine the location of active PF synapses. PF synapses are oriented perpendicular to the plane of the Purkinje cell dendritic tree; in the parasagittal slices utilized in our experiments, PFs are perpen-

dicular to the horizontal surface of the slice. Such an arrangement makes it possible to activate a variable number of PF synapses simply by placing an extracellular stimulation pipette on the surface of the slice. In such conditions, it was fairly easy to identify the site of PF activity by monitoring the Ca^{2+} transient associated with the PF stimulation.

A short train of PF responses evoked by burst stimulation reliably produced localized dendritic Ca^{2+} signals (Fig. 3C,D), even at stimulus intensities too weak to generate dendritic Ca^{2+} signals during single stimuli (Fig. 3A,B). These Ca signals were prevented by CNQX, suggesting that burst stimuli cause summation of local, AMPA receptor-mediated depolarizations that activate voltage-gated calcium channels. The Ca^{2+} response to a single burst stimulus was therefore used to identify the site of active PF synapses. This stimulus did not depress synaptic transmission (not shown). Ca^{2+} signals associated with PF activation could be de-

Figure 3: Identification of the synaptic input area. (A) EPSC (lower trace) evoked by single-shock PF stimulation. No accompanying change in the Ca^{2+} -dependent dendritic fluorescence was detected (upper trace). The arrowhead denotes the time of PF stimulation. (B) Pseudocolor image of the fluorescence during this single-shock PF stimulation. The dashed box indicates the region in which the dendritic fluorescence was averaged for the traces shown in A, C, and Fig. 4A. (C) EPSC and Ca^{2+} -dependent fluorescence transient evoked by a brief burst stimulation. (D) Pseudocolor image showing the spatial extent of the Ca^{2+} signal during the burst stimulation.

tected 20–30 min after establishing the whole-cell configuration. Excitatory synaptic currents produced by PF activity (PF-EPSCs) were then recorded for ~10–15 min (see below) to establish the baseline level of PF transmission. Measurements of Purkinje cell Ca^{2+} levels were begun just before applying LTD-inducing stimuli. These measurements ensured that PF responses to single stimuli were not accompanied by a Ca^{2+} transient and also served to quantify the spatiotemporal extent of the Ca^{2+} transient produced by the burst stimulation (Fig. 3C,D). Control experiments demonstrated that imaging of PF-evoked Ca^{2+} signals did not interfere with the induction of LTD (not shown).

TRANSIENT AND LOCAL ELEVATION OF DENDRITIC Ca^{2+} DURING INDUCTION OF LTD

The spatial and temporal dimensions of dendritic Ca^{2+} signals resulting from single bouts of PF activity are well characterized (Fig. 3; Denk et al. 1995; Eilers et al. 1995a, Hartell 1996a). However, the degree of localization of Ca^{2+} signals that accompany the generation of LTD by intense or repeated PF activity is not known. The high spatial and temporal resolution of our confocal imaging method allowed us to characterize PF-evoked Ca^{2+} signals, within the dendritic arbor of individual Purkinje cells, during induction of LTD.

The patterns of PF stimulation that were sufficient to induce LTD produced substantial increases in dendritic $[\text{Ca}^{2+}]_i$ that were sustained throughout the stimulus period and then decayed back to baseline. In the example shown in Figure 4, which is taken from the same experiment as Figure 3, LTD was induced by a single burst stimulus (50 Hz) given repetitively at 1 Hz for 90 sec. Following this period of stimulation, the amplitude of PF-EPSCs decreased over ~10 minutes and then stabilized at 60% of its initial amplitude. The depression in the peak amplitude of PF-EPSCs was not accompanied by a significant change in EPSC time course (Fig. 4A).

During the induction period, the dendritic Ca^{2+} signals reached a plateau level within the first three to four burst stimuli and remained at this level until the end of the stimulus train (Fig. 4C). The plateau in fluorescence intensity may have been attributable to saturation of the high-affinity indicator dye, which has a K_d of ~200 nM. Although we may not have detected the peak Ca^{2+} levels achieved during the train of stimuli, these

Figure 4: Elevated dendritic Ca^{2+} concentration during the induction of LTD. (A) Averages of EPSCs recorded directly before, during, and 20 min after LTD induction. The traces labeled before/after show the averaged response from before and after, superimposed for comparison. EPSC records are averages of six responses, except for the EPSC labeled during induction, which is the average of all responses during the induction period. Stimulation artifacts were reduced for clarity. (B) Time course of EPSC peak amplitudes during the LTD experiment. Values are normalized with respect to the mean baseline value. Except for during the induction period, PFs were activated with single-shock stimulations at 0.1 Hz. Individual data points are shown, with no binning. The horizontal bar indicates the induction period (corresponding to the horizontal bar in C). LTD was induced by short 50-Hz trains of PF stimulation given at 1 Hz for 90 sec. (C) Time course of the Ca^{2+} -dependent dendritic fluorescence during the induction period. For measurement, fluorescence images were averaged over a period of 500 msec and acquired at ~0.4 Hz. The dendritic Ca^{2+} signal was averaged in the same dendritic compartment as in Fig. 3B. Note the slow decay time of the fluorescence signal at the end of the induction period.

Ca^{2+} signals were clearly much larger than those produced by a single burst stimulation (cf. Fig. 3 with Figs. 4 and 5). When PF stimulation was halted, fluorescence intensity returned to its baseline level with a time constant of ~15 sec. This decay time was substantially prolonged compared to that evoked by a single burst of stimuli or a single strong stimulus (see Eilers et al. 1995a; Har-

tell 1996a), both of which decayed with a time constant of ~ 2 sec (Fig. 3C). These prolonged and large increases in dendritic Ca^{2+} levels in Ca^{2+} levels were observed in a total of five experiments employing burst stimuli; in every case, LTD was induced by these stimuli. In contrast, in three other experiments where a train of burst stimuli a train failed to produce a prolonged buildup of dendritic Ca^{2+} or a larger Ca signal than that produced by a single burst, no depression of PF transmission was induced. These correlations indicate that the dendritic Ca^{2+} signals are necessary to induce LTD.

The repetitive stimulation of PFs during induction of LTD yielded Ca^{2+} signals (Fig. 5) that were restricted to the same dendritic compartment that was activated during single bursts of PF activity (Fig. 3). For both types of PF activity, the Ca^{2+} increase was localized to the same set of terminal dendrites and connecting parent dendrites. Thus, even during prolonged Ca^{2+} transients there was no significant spread of the signal to neighboring dendritic branches. These results indicate that very effective compartmentation mechanisms restrict the dendritic movement of Ca^{2+} . Our finding that these highly localized increases in dendritic $[\text{Ca}^{2+}]_i$ provide a sufficient Ca^{2+} signal to induce LTD provides a functional role for local PF-evoked Ca^{2+}

signals and confirms the idea that the terminal dendritic branches of Purkinje cells act as functional signaling units (Eilers et al. 1995a).

Discussion

The major finding of this study is that induction of PF-LTD is associated with a transient elevation of $[\text{Ca}^{2+}]_i$ that is restricted to the spiny dendritic area activated by a beam of PFs. Although these Ca^{2+} signals persisted throughout the conditioning stimulus, they recovered to baseline values in less than a minute and did not spread to distant dendrites. These findings, combined with the observations that intracellular Ca^{2+} chelators prevent the induction of PF-LTD (Hartell 1996a), indicate that localized increases in dendritic $[\text{Ca}^{2+}]_i$ can provide a Ca^{2+} signal sufficient to induce LTD. Such local Ca^{2+} signals have been reported before, both in Purkinje cells (Denk et al. 1995; Eilers et al. 1995a; Hartell 1996a) and in hippocampal pyramidal cells (Magee et al. 1996). While it has been proposed that these signals play a role in dendritic integration, our study is the first to unambiguously assign a functional role to these signals.

The important Ca^{2+} signal for the induction of

Figure 5: Spatial extent of the dendritic Ca^{2+} transient during induction of LTD. Pseudocolor images of the relative increase in Ca^{2+} -dependent fluorescence during the induction of PF-LTD. Same experiment as in Fig. 4. The indicated time points refer to the time axis in Fig. 4C.

PF-LTD thus appears to be a transient and highly localized buildup of Ca^{2+} in the vicinity of active PF synapses. Because a high-affinity Ca^{2+} indicator was used to measure postsynaptic Ca^{2+} signals, Ca^{2+} concentration at the local sites of active PFs may have been underestimated because of dye saturation. Thus, our data do not allow us to quantify the Ca^{2+} concentrations needed for the triggering of the LTD. Nonetheless, we can conclude that Ca^{2+} levels return to baseline levels rapidly (in <1 min) following the PF activity that induces LTD. Even though postsynaptic Ca^{2+} concentration rises only transiently, PF-LTD develops gradually over the subsequent 10–20 min. Thus, in terms of kinetic properties, the Ca^{2+} -dependent induction of LTD by PF activity closely resembles the induction of LTD produced by CF activation or by Purkinje cell depolarization even though the Ca^{2+} signals produced by Purkinje cell depolarization or by CF activity are more spatially widespread (Konnerth et al. 1992). At present it is not clear whether these various LTD-inducing stimuli yield different Ca^{2+} signals at the PF synapses. For example, there are indirect indications of supralinear postsynaptic signal summation during coactivation of CFs and PFs (Batchelor and Garthwaite 1997).

Although it is clear that PF activity capable of producing LTD is accompanied by a rise in dendritic $[\text{Ca}^{2+}]_i$, the site of the coincidence detector for signal integration remains to be determined. Although we did not measure the concentration of Ca^{2+} in individual dendritic spines in the present experiments, in an earlier study we demonstrated that PF activity increases Ca^{2+} levels in both the dendritic shaft and spines (Eilers et al. 1995a). It is therefore likely that induction of LTD is accompanied by sustained increases in Ca^{2+} concentration in both dendritic shafts and spines. Further work will be needed to determine whether the biochemical machinery involved in LTD induction requires the buildup of Ca^{2+} in the dendritic shaft, spines, or both. A related open question is whether there is a threshold in $[\text{Ca}^{2+}]_i$ for induction of LTD or whether graded synaptic activity, and the associated graded Ca^{2+} signals (Eilers et al. 1995a), produce proportional reductions in the efficacy of synaptic transmission of PFs.

Various Ca^{2+} sources may contribute to the signals occurring during the induction of PF-LTD. Both AMPA-type glutamate receptor channels (AMPA-Rs) and metabotropic glutamate receptors (mGlu-Rs) are activated during PF synaptic transmission (Konnerth et al. 1990; Batchelor et al.

1994). Ca^{2+} influx through postsynaptic AMPA-Rs has been implicated in a form of synaptic plasticity in dorsal root neurons (Gu et al. 1996). However, because Purkinje cell AMPA-Rs have a very low permeability to Ca^{2+} (Tempia et al. 1996), it is unlikely that Ca^{2+} influx through these receptors plays a significant role in PF-LTD. It is more likely that AMPA-R activation promotes Ca^{2+} influx by activating dendritic voltage-gated Ca^{2+} channels (VGCCs). Both T-type (Mouginot et al. 1997) and P-type (Regan 1991; Usowicz et al. 1992) VGCCs are present in Purkinje cell dendrites; the precise roles of these Ca^{2+} channel subtypes in PF-mediated Ca^{2+} signaling and in the induction of PF-LTD remains to be determined. Activation of type-I mGlu-Rs, the subtype located at PF-Purkinje cell synapses, produces IP_3 . Thus, Ca^{2+} release by IP_3 (Llano et al. 1991b; E.A. Finch and G.J. Augustine, unpubl.), as well as Ca^{2+} -induced Ca^{2+} release (Kano et al. 1995), may also contribute to the local dendritic Ca^{2+} signals that induce PF-LTD.

The coincidence detection mechanism for CF and depolarization-mediated LTD requires back-propagation of electrical activity into dendrites. In contrast, we have found that PF-LTD can be induced in the absence of somatic regenerative electrical activity. PF-LTD is instead a purely dendritic form of integration, based on the coincidence of PF activity and a concomitant local increase in dendritic $[\text{Ca}^{2+}]_i$. PF-LTD thus represents a striking example of local dendritic signal processing in the mammalian brain.

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References

- Aiba, A., M. Kano, C. Chen, M.E. Stanton, G.D. Fox, K. Herrup, T.A. Zwingman, and S. Tonegawa. 1994. Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* **79**: 377–388.
- Batchelor, A. and J. Garthwaite. 1997. Frequency detection and temporally dispersed synaptic signal association through

- a metabotropic glutamate receptor pathway. *Nature* **385**: 74–77.
- Batchelor, A.M., D.J. Madge, and J. Garthwaite. 1994. Synaptic activation of metabotropic glutamate receptors in the parallel fibre-Purkinje cell pathway in rat cerebellar slices. *Neuroscience* **63**: 911–915.
- Bourne, H.R. and R. Nicoll. 1993. Molecular machines integrate coincident synaptic signals. *Cell* **72**: 65–75.
- Crepel, F. and D. Jaillard. 1991. Pairing of pre- and postsynaptic activities in cerebellar Purkinje cells induces long-term changes in synaptic efficacy in vitro. *J. Physiol.* **432**: 123–141.
- Crepel, F. and M. Krupa. 1988. Activation of protein kinase C induces a long-term depression of glutamate sensitivity of cerebellar Purkinje cells. An in vitro study. *Brain Res.* **458**: 397–401.
- Denk, W., M. Sugimori, and R. Llinás. 1995. Two types of calcium responses limited to single spines in cerebellar Purkinje cells. *Proc. Natl. Acad. Sci.* **92**: 8279–8282.
- De Schutter, E. 1995. Cerebellar long-term depression might normalize excitation of Purkinje cells: a hypothesis. *Trends Neurosci.* **18**: 291–295.
- De Schutter, E. and R. Maex. 1996. The cerebellum: cortical processing and theory. *Curr. Opin. Neurobiol.* **6**: 759–764.
- Edwards, F., A. Konnerth, B. Sakmann, and T. Takahashi. 1989. A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflügers Arch.* **414**: 600–612.
- Eilers, J., G.J. Augustine, and A. Konnerth. 1995a. Subthreshold synaptic Ca^{2+} signalling in fine dendrites and spines of cerebellar Purkinje neurons. *Nature* **373**: 155–158.
- Eilers, J., R. Schneggenburger, and A. Konnerth. 1995b. Patch clamp and calcium imaging in brain slices. In *Single channel recording* (ed. B. Sakmann, and E. Neher), pp. 213–229. Plenum Publishing Co., New York, NY.
- Gu, J., C. Albuquerque, C. Lee, and A. MacDermott. 1996. Synaptic strengthening through activation of Ca^{2+} -permeable AMPA receptors. *Nature* **381**: 793–796.
- Hartell, N.A. 1994. Induction of cerebellar long-term depression requires activation of glutamate metabotropic receptors. *NeuroReport* **5**: 913–916.
- . 1996a. Strong activation of parallel fibers produce localized calcium transients and a form of LTD that spreads to distant synapses. *Neuron* **16**: 601–610.
- . 1996b. Inhibition of cGMP breakdown promotes the induction of cerebellar long-term depression. *J. Neurosci.* **16**: 2881–2890.
- Ito, M. 1984. *The cerebellum and neuronal control*. Raven Press, New York, NY.
- . 1989. Long-term depression. *Annu. Rev. Neurosci.* **12**: 85–102.
- . 1991. The cellular basis of cerebellar plasticity. *Curr. Opin. Neurobiol.* **1**: 616–620.
- Ito, M. and M. Kano. 1982. Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex. *Neurosci. Lett.* **33**: 253–258.
- Ito, M., M. Sakurai, and P. Tongroach. 1982. Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *J. Physiol.* **324**: 113–134.
- Kano, M., O. Garaschuk, A. Verkhratsky, and A. Konnerth. 1995. Ryanodine receptor-mediated intracellular calcium release in rat cerebellar Purkinje neurones. *J. Physiol.* **487.1**: 1–16.
- Kasono, K. and T. Hirano. 1994. Critical role of postsynaptic calcium in cerebellar long-term depression. *NeuroReport* **6**: 17–20.
- . 1995. Involvement of inositol trisphosphate in cerebellar long-term depression. *NeuroReport* **6**: 569–572.
- Konnerth, A., I. Llano, and C.M. Armstrong. 1990. Synaptic currents in cerebellar Purkinje cells. *Proc. Natl. Acad. Sci.* **87**: 2662–2665.
- Konnerth, A., J. Dreessen, and G.J. Augustine. 1992. Brief dendritic calcium signals initiate long-lasting synaptic depression in cerebellar Purkinje cells. *Proc. Natl. Acad. Sci.* **89**: 7051–7055.
- Konnerth, A., R.Y. Tsien, K. Mikoshiba, and J. Altman. 1996. *Coincidence detection in the nervous system*. Human Frontier Science Program, Strasbourg, France.
- Krupa, D.J., J.K. Thompson, and R.F. Thompson. 1993. Localization of a memory trace in the mammalian brain. *Science* **260**: 989–991.
- Lev-Ram, V., L. Makings, P. Keitz, J. Kao, and R. Tsien. 1995. Long-term depression in cerebellar Purkinje neurons results from coincidence of nitric oxide and depolarization-induced Ca^{2+} transients. *Neuron* **15**: 407–415.
- Linden, D.J. 1994. Long-term synaptic depression in the mammalian brain. *Neuron* **7**: 457–472.
- Linden, D.J. and J.A. Connor. 1991. Participation of postsynaptic PKC in cerebellar long-term depression in culture. *Science* **254**: 1656–1659.
- Linden, D.J., M.H. Dickinson, M. Smeyne, and J.A. Connor. 1991. A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. *Neuron* **7**: 81–89.

Eilers et al.

Llano, I., A. Marty, C.M. Armstrong, and A. Konnerth. 1991a. Synaptic- and agonist-induced excitatory currents of Purkinje cells in rat cerebellar slices. *J. Physiol.* **434**: 183–213.

Llano, I., J. Dreessen, M. Kano, and A. Konnerth. 1991b. Intradendritic release of calcium induced by glutamate in cerebellar Purkinje cells. *Neuron* **7**: 577–583.

Magee, J.C., G. Christofi, H. Miyakawa, B. Christie, N. Lasser-Ross, and D. Johnston. 1996. Subthreshold synaptic activation of voltage-gated Ca^{2+} channels mediates a localized Ca^{2+} influx into the dendrites of hippocampal pyramidal neurons. *J. Neurophysiol.* **74**: 1335–1342.

Miyakawa, H., V. Lev-Ram, N. Lasser-Ross, and W.N. Ross. 1992. Calcium transients evoked by climbing fiber and parallel fiber synaptic inputs in Guinea pig cerebellar Purkinje neurons. *J. Neurophysiol.* **68**: 1178–1189.

Mouginot, D., J.-L. Bossu, and B.H. Gähwiler. 1997. Low-threshold Ca^{2+} currents in dendritic recordings from Purkinje cells in rat cerebellar slice cultures. *J. Neurosci.* **17**: 160–170.

Neher, E and G.J. Augustine. 1992. Calcium gradients and buffers in bovine chromaffin cells. *J. Physiol.* **450**: 273–301.

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

Regan, L.J. 1991. Voltage-dependent calcium currents in Purkinje cells from rat cerebellar vermis. *J. Neurosci.* **11**: 2259–2269.

Rexhausen, U. 1992. "Bestimmung der Diffusionseigenschaften von Fluoreszenzfarbstoffen in verzweigten Nervenzellen unter Verwendung eines rechnergesteuerten Bildverarbeitungssystems." Ph.D. Thesis, University of Göttingen, Germany.

Ross, W.N. and R. Werman. 1987. Mapping calcium transients in the dendrites of Purkinje cells from the Guinea-pig cerebellum in vitro. *J. Physiol.* **389**: 319–336.

Sakurai, M. 1990. Calcium is an intracellular mediator of the climbing fiber in induction of cerebellar long-term depression. *Proc. Natl. Acad. Sci.* **87**: 3383–3385.

Tempia, F., M. Kano, R. Schneggenburger, C. Schirra, O. Garaschuk, T. Plant, and A. Konnerth. 1996. Fractional calcium current through neuronal AMPA-receptor channels with a low calcium permeability. *J. Neurosci.* **16**: 456–466.

Usovich, M.M., M. Sugimori, B. Cherksey, and R. Llinás. 1992. P-type calcium channels in the somata and dendrites of adult cerebellar Purkinje cells. *Neuron* **9**: 1185–1199.

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