RESEARCH

The Role of Dendritic Action Potentials and Ca^{2+} Influx in the Induction of Homosynaptic Long-term Depression in Hippocampal CA1 Pyramidal Neurons

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Abstract

Long-term depression (LTD) of synaptic efficacy at CA1 synapses is believed to be a Ca^{2+}-dependent process. We used high-speed fluorescence imaging and patch-clamp techniques to quantify the spatial distribution of changes in intracellular Ca^{2+} accompanying the induction of LTD at Schaffer collateral synapses in CA1 pyramidal neurons. Low-frequency stimulation (3 Hz), which was subthreshold for action potentials, produced small changes in $[Ca^{2+}]_i$ and failed to elicit LTD. Increasing the stimulus strength so that action potentials were generated produced both robust LTD and increases in $[Ca^{2+}]_i$. Back-propagating action potentials at 3 Hz in the absence of synaptic stimulation also produced increases in $[Ca^{2+}]_i$, but failed to induce LTD. When subthreshold synaptic stimulation was paired with back-propagating action potentials, however, large increases in $[Ca^{2+}]_i$ were observed and robust LTD was induced. The LTD was blocked by the *N*-methyl-D-aspartate receptor (NMDAr) antagonist APV, and stimulus-induced increases in $[Ca^{2+}]_i$ were reduced throughout the neuron under these conditions. The LTD was also dependent on Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCCs), because LTD was severely attenuated or blocked by both nimodipine and Ni^{2+}. These findings suggest that back-propagating action potentials can exert a powerful control over the induction of LTD and that both VGCCs and NMDArs are involved in the induction of this form of plasticity.

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Introduction

Long-term depression (LTD) and long-term potentiation (LTP) refer to protracted decreases and increases in synaptic efficacy, respectively, and together these two phenomena are believed to be involved in certain forms of mnemonic processing. Although there are a number of paradigms that can result in long-lasting reductions in synaptic efficacy, low-frequency stimulation (LFS) at 1–5 Hz recently has been used to reliably induce a robust LTD in vitro. This form of LTD has been termed homosynaptic LTD because it occurs only at those synapses that are active during the induction procedure (Dudek and Bear 1992; Mulkey and Malenka 1992; Bolshakov and Siegelbaum 1994). Like LTP in the CA1 region, homosynaptic LTD can exhibit input specificity, reversibility, saturation, and frequency-dependency (Dudek and Bear 1992, 1993).

Research into the mechanisms responsible for the genesis of LTD has led to the hypothesis that an increase in Ca^{2+} in the postsynaptic neuron plays an important role in the induction phase (Dudek and Bear 1992; Mulkey and Malenka 1992; Bolshakov and Siegelbaum 1994; Selig et al. 1995). More specifically, it has been proposed that LTD and LTP require different levels of $[Ca^{2+}]_i$ for their induction, with LTP requiring a greater influx than

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LTD (Christie et al. 1994; Larkman and Jack 1995; Linden and Connor 1995). To date, however, no direct recordings of Ca^{2+} activity actually have been made during the induction of homosynaptic LTD, and the main evidence for Ca^{2+} involvement comes from the fact that reducing either $[Ca^{2+}]_i$ levels or Ca^{2+} influx prevents LTD (Mulkey and Malenka 1992).

It is known that Na^+-dependent action potentials that back-propagate into the apical dendrites of CA1 neurons lead to widespread increases in $[Ca^{2+}]_i$ (Jaffe et al. 1992; Miyakawa et al. 1992) via influx through voltage-gated Ca^{2+} channels (VGCCs; Christie et al. 1995). These back-propagating action potentials may also open *N*-methyl-D-aspartate receptors (NMDArs) by alleviating the Mg^{2+} block and producing localized entry of Ca^{2+} (Mayer and Westbrook 1987; Spruston et al. 1995a). Several studies have suggested that the NMDAr is essential for the induction of homosynaptic LTD (Dudek and Bear 1992; Mulkey and Malenka 1992; Selig et al. 1995), although others have reached different conclusions (Wexler and Stanton 1993; Bolshakov and Siegelbaum 1994). Similarly, evidence for (Bolshakov and Siegelbaum 1994) and against (Mulkey and Malenka 1992; Selig et al. 1995) the involvement of at least the L-type of VGCC in homosynaptic LTD has also been reported. The putative involvement of other VGCC subtypes in hippocampal LTD remains to be examined.

Fluorescence imaging studies have indicated that several types of VGCCs are located throughout the dendrites, where they may play an active role in synaptic plasticity (Regehr and Tank 1990; Jaffe et al. 1992; Miyakawa et al. 1992; Regehr and Tank 1992; Christie et al. 1995). Whereas the overall density of Ca^{2+}-channels does not appear to change in more distal dendritic regions (Magee and Johnston 1995), the relative contribution of the different channel subtypes to Ca^{2+} influx differs between somatic and dendritic regions (Christie et al. 1995). Low-voltage-activated (LVA) T-type channels have been shown to be activated by synaptically generated excitatory postsynaptic potentials (EPSPs) in CA1 pyramidal cells (Magee et al. 1995), indicating that they can provide a source of Ca^{2+} in the absence of back-propagating action potentials. At present, however, it appears that an increase in Ca^{2+} offers little predictive value for the induction of either LTD or LTP (Neveu and Zucker 1996). Therefore, it remains unclear whether there is actually some threshold level of Ca^{2+} that determines the induction of LTD or LTP, or whether the activation of certain channel subtypes and Ca^{2+} localization might serve as indicators for the form of plasticity to be expressed. In the present report, we use fura-2 fluorescence imaging to measure the changes in $[Ca^{2+}]_i$ in soma and dendrites during various LTD induction paradigms. We found that LFS paired with back-propagating action potentials was necessary for the induction of LTD under our conditions. Furthermore, LTD induction was prevented by both D,L-2-amino-5-phosphonovaleric acid (APV) and blockers of certain VGCC subtypes. In addition, the increase in $[Ca^{2+}]_i$ during the induction phase was reduced in both soma and dendrites in the presence of these agents.

Materials and Methods

HIPPOCAMPAL SLICE PREPARATION

Hippocampal slices were prepared from young Sprague-Dawley rats (12–28 days). Rats were decapitated and the brain was rapidly dissected in cold saline composed of (in mM): 124 NaCl, 26 $NaHCO_3$, 10 dextrose, 2.5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$, and 1.25 NaH_2PO_4 superfused with 95% O_2 and 5% CO_2. Serial slices (400 μm) were taken and placed in an oxygenated holding chamber where they were incubated at 32°C for at least 20 min before being stored at room temperature for the remainder of the experiment (<6 hr).

ELECTROPHYSIOLOGICAL AND FLUORESCENCE IMAGING METHODS

Electrophysiological recordings were carried out at 32°C by use of conventional patch-clamp whole-cell recording techniques. Individual slices were transferred as needed to a submerged holding chamber, and a ×40 water-immersion objective and differential interference contrast (DIC) optics were used to view the slice. Light in the near infrared range (740 nm) was used to better resolve individual neurons and their dendritic arbors. Whole-cell recording pipettes (2–4 MΩ) were pulled from borosilicate glass (Drummond) and filled with (mM): 120 KGluconate, 20 KCl, 10 HEPES, 4 NaCl, 4 Mg-ATP, 0.3 Mg-GTP, 14 phosphocreatine (pH 7.25, KOH), and 0.16 fura-2. Whole-cell patch-clamp recordings were made from visually identified CA1 pyramidal neurons lo-

cated within 50 μm of the slice surface with an Axoclamp 2A amplifier in "bridge" mode (Axon Instruments). Neurons exhibited a resting membrane potential (Vm) between −60 and −70 mV. A single, etched tungsten wire (tip diameter <5 μm) was placed near the dendrites of the cell under study (~100–200 μm from the soma) for focal extracellular stimulation. Electrode series resistance was monitored in all recordings.

By use of a cooled CCD camera (Photometrics, Tucson, AZ) in sequential frame transfer mode, high-speed fluorescence images (25-ms frame intervals) were recorded from a 220 μm length of soma and dendrite. Figure 1 presents a schematic of the recording and stimulation arrangement used in the whole-cell experiments. Relative changes in $[Ca^{2+}]_i$ were quantified as changes in ΔF/F, where F is fluorescence intensity before stimulation (after subtracting autofluorescence) and ΔF is the reduction from this value during neuronal activity. The bleaching correction was determined by taking a fluorescence measurement of the neuron under nonstimulated conditions. The tissue autofluorescence was determined by making an equivalent measurement at a parallel location in the slice away from the dye-filled neuron at the end of the experiment (Jaffe et al. 1992; Miyakawa et al. 1992). With the ×40 objective we were able to image simultaneously only the soma and the first 150–200 μm of the proximal dendrites. Within this field of interest, three locations were chosen for analysis: the soma, the first 25 μm of the proximal dendrite (proximal), and an intermediate region of the dendrites about 100–150 μm from the soma (midradiatum). These regions are indicated in the figures.

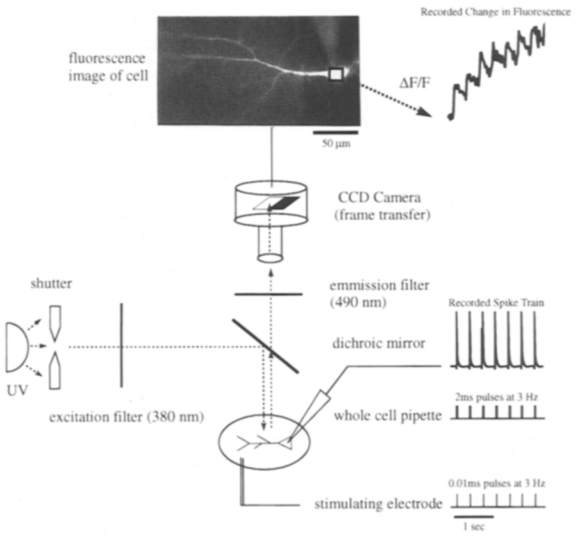

Figure 1: Schematic of optical and electrical recording arrangements. Individual cells were filled with an internal solution containing the fluorescent dye fura-2 (80–160 μM) via a whole-cell pipette, allowing simultaneous optical and electrical recordings to be taken.

Stable electrical recordings were required for a minimum of 10 min before beginning the induction protocols, and recordings were continued for at least another 30–60 min. LTD was induced by applying 900 pulses at 3 Hz through the radiatum stimulating electrode. The 900 pulses were administered in epochs of 30 to 60 sec, with intervals of 5 sec between epochs, until a total of 900 pulses was reached. During each epoch, the fluorescence was measured for the first 5–10 sec of the stimulation. For each induction procedure, the reported changes in ΔF/F are based on measurements of the peak fluorescence at the end of the fluorescence measurement interval averaged over all of the epochs. All electrical data in the text are presented as the mean ± S.E.M. of 24 recordings taken over a 6-min period 30 min following the induction procedure. Data were analyzed with one sample and unpaired *t*-tests, as appropriate. The slope of the rising phase of the EPSP was used as the dependent measure for quantifying electrophysiological data. APV [50 μM; Research Biochemicals International (RBI)] and $NiCl_2$ (25 μM; Sigma) were prepared daily from 5 mM stock solutions. Nimodipine (10 μM; RBI) was also prepared daily in ethanol, and recordings were carried out in a darkened room.

Results

STIMULUS REQUIREMENTS FOR LTD IN WHOLE-CELL RECORDINGS

The degree of postsynaptic depolarization necessary for the induction of homosynaptic LTD remains unclear at present. It is a potentially important parameter, however, because the magnitude of the response produced by the conditioning stimulus can influence greatly the nature of the postsynaptic Ca^{2+} signal. For example, stimuli that fail to elicit action potentials produce only local rises in $[Ca^{2+}]_i$ that are primarily caused by the activation of T-type Ca^{2+} channels (Magee et al. 1995). The generation of action potentials by the stimuli, on the other hand, alters the magnitude and spatial distribution of the Ca^{2+} signals. In response to each action potential generated, back-

propagating action potentials course through the dendrites and produce widespread increases in $[Ca^{2+}]_i$ as L-, N-, P-, and R-type channels are activated (Christie et al. 1995). In the present experiments, the application of 3-Hz stimuli subthreshold for the generation of action potentials (10–20 mV EPSPs) failed to induce a significant degree of LTD ($-6.1\pm10.5\%$; $n=4$; $P>0.05$) (Fig. 2). As expected from previous imaging experiments, only a small change in $[Ca^{2+}]_i$ was found to accompany the subthreshold synaptic stimulation (soma: $3.3\pm2.2\%$ $\Delta F/F$; proximal: $2.0\pm0.8\%$ $\Delta F/F$; mid-radiatum: $1.8\pm0.8\%$ $\Delta F/F$; Fig. 2A).

Increasing the strength of the synaptic stimulation so that action potentials could be elicited in the postsynaptic cell during the 3-Hz stimulation produced robust LTD that lasted for at least 30 min ($-40.8\pm6.3\%$; $n=5$; $P<0.05$; Fig. 3). Suprathreshold stimulation also produced large rhythmic Ca^{2+} transients throughout the soma and proximal dendritic tree during the induction procedure. The change in fluorescence over time rose to a maximum value over the initial 2–4 sec of each period of conditioning stimulation, and then reached a plateau for the remainder of the acquisition period (soma: $9.5\pm2.1\%$ $\Delta F/F$; proximal: $8.7\pm1.9\%$ $\Delta F/F$; mid-radiatum: $10.6\pm4.8\%$ $\Delta F/F$; Fig. 3A). Past studies have indicated that these transients are reflective of VGCC activity in response to action potentials back-propagating through the dendrites (Miyakawa et al. 199; Christie et al. 1995).

Previous studies have shown that a large transient increase in Ca^{2+} in the absence of synaptic stimulation does not produce stable LTP (Kullmann et al. 1992), although under special conditions, LTD may be induced by a rise in postsynaptic $[Ca^{2+}]_i$ alone without concurrent synaptic transmission (Christofi et al. 1993). To test this, we also produced an increase in postsynaptic Ca^{2+} by generating back-propagating action potentials in CA1 neurons. Injections of depolarizing current (1–2 ms; sufficient to generate action potentials) were administered via the whole-cell pipette to induce action potentials at 3 Hz without concurrent synaptic stimulation. Large Ca^{2+} transients were observed in response to the depolarizing pulses (soma: $22.8\pm0.3\%$ $\Delta F/F$; proximal: $25.0\pm0.3\%$ $\Delta F/F$; mid-radiatum: $21.4\pm0.9\%$ $\Delta F/F$; Fig. 4A). In these cells, however, the generation of 900 action potentials alone failed to induce LTD of synaptic responses ($9.53\pm16.8\%$; $n=6$; $P>0.05$; Fig. 4B). The Ca^{2+} transients could be blocked by adding Cd^{2+} (100–200 μM) to the external bathing medium, suggesting that they occurred in response to Ca^{2+} entry via VGCCs (data not shown).

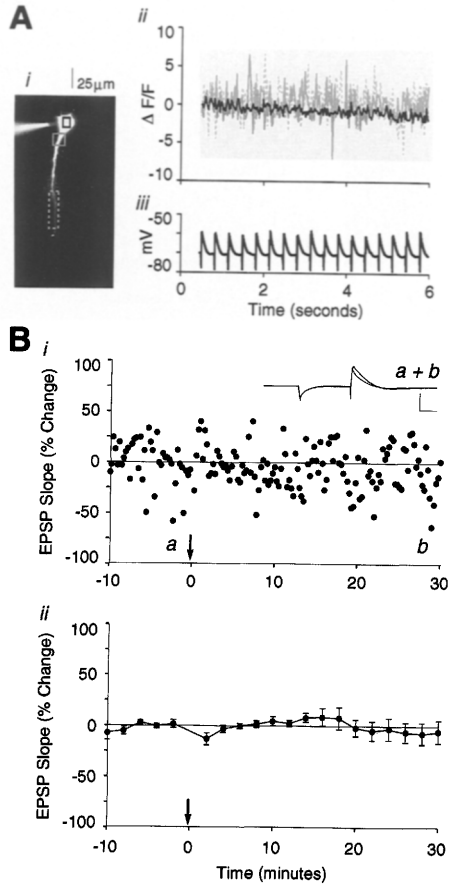

Figure 2: EPSPs, subthreshold for action potential generation, fail to induce LTD in whole cell recordings. (*Ai*) Positive photomicrograph of a fura-2-filled CA1 pyramidal neuron. Boxes indicate regions used in the final analysis for the cell shown. (*Aii*) Only small Ca^{2+} transients were apparent during the application of the low-frequency trains of EPSPs shown in Aiii. (*Aiii*) 10+ mV EPSPs evoked during the course of administering 900 pulses at 3 Hz. The amplitude of the EPSPs did not vary significantly when EPSPs were administered in this fashion. (*Bi*) Individual EPSP slopes recorded for 10 min prior to and 30 min following the application of the 3-Hz conditioning stimuli. Inset shows averaged responses recorded over the (*a*) 5 min prior to and (*b*) 30 min following the application of the conditioning stimuli in the cell shown in A. (*Bii*) Average responses from four slices administered the subthreshold pulses at 3 Hz. Stimulation of the presynaptic pathway at 3 Hz produced 10–15 mV EPSPs over the course of the stimulus protocol in all cells tested. Arrows indicate time point at which the low-frequency stimulation was administered. Scale bars: 10 mV, 100 ms.

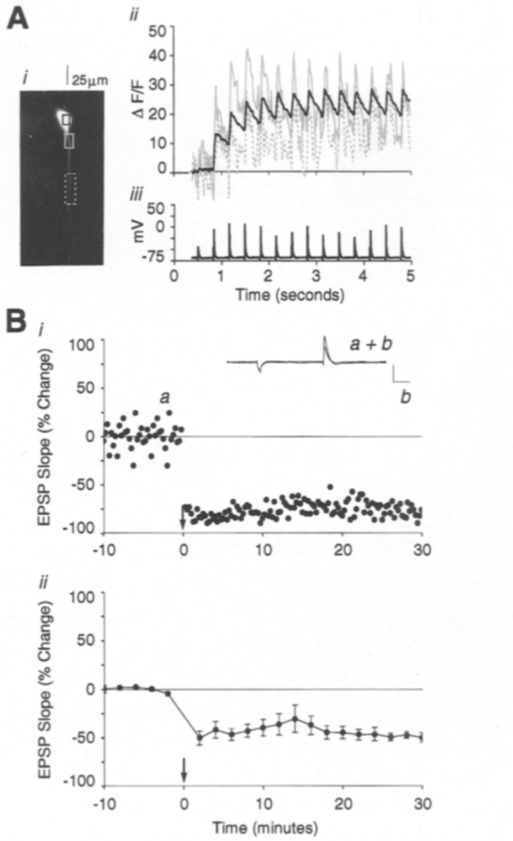

Figure 3: Suprathreshold synaptic stimulation elicits homosynaptic LTD. (*Ai*) Positive photomicrograph of a fura-2-filled CA1 pyramidal neuron from which data in *A* and *Bi* are presented. (*Aii, Aiii*) Moderate increases in the Ca^{2+} transients recorded during the 3-Hz stimulation are apparent in the soma, and the proximal and distal dendrites of recorded cells. Note the minimal change in fluorescence that accompanies the first pulse in the 3-Hz train (which does not cause an action potential in the postsynaptic neuron). (*Bi*) LTD of EPSP slopes is apparent in recordings from individual neurons following the application of the suprathreshold stimuli. (*Bii*) Averaged data taken from all slices which were administered the suprathreshold conditioning paradigm ($n = 6$). Arrows indicate time at which the low-frequency stimulation was administered. Scale bars: 10 mV, 100 ms.

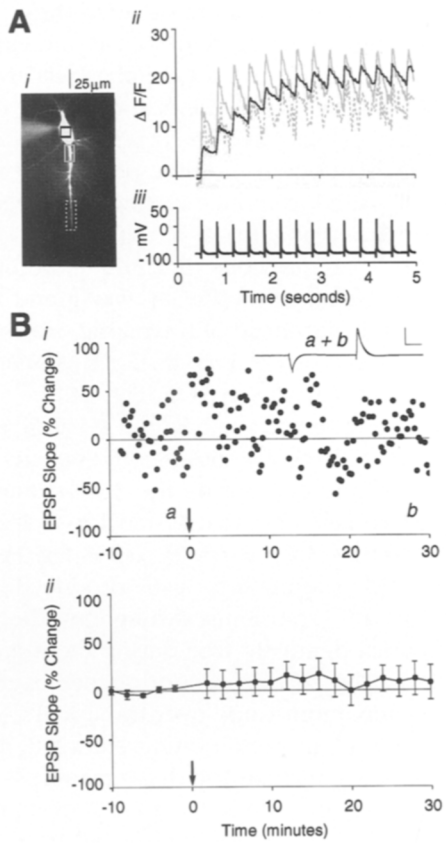

Figure 4: Postsynaptically generated action potentials alone are not sufficient to induce LTD. (*Ai*) Positive photomicrograph of a fura-2-filled CA1 pyramidal neuron from which data in *A* and *Bi* are presented. (*Aii*) Moderate changes in fluorescence accompany each action potential (*Aiii*) induced by the administration of a short depolarizing pulse (1–2 ms) via the whole cell pipette. (*Bi*) Presentation of 900 depolarizing pulses at 3 Hz fails to induce LTD of single evoked responses. (*Bii*) Average change in evoked responses from four neurons that received the low-frequency current injections alone. Arrows indicate time point at which the low-frequency stimulation was administered. Scale bars: 10 mV, 100 ms.

Given that suprathreshold synaptic stimulation (synaptic transmission and the generation of action potentials) induced a robust LTD, we decided to utilize a more controlled paradigm in which action potentials elicited with depolarizing current injections to the soma could be paired with subthreshold synaptic stimulation. In this way, we could test whether the generation of action potentials or strong synaptic stimulation were critical for the LTD induction. Whereas both back-propagating action potentials and subthreshold synaptic stimulation failed to induce LTD when administered alone, their conjunctive application resulted in substantial LTD ($-48.7 \pm 4.0\%$; $n = 5$; $P > 0.05$; Fig. 5) that could be recorded for up to 1 hr ($-66.3 \pm 15.4\%$; $n = 2$). The degree of depression produced by the pairing paradigm did not differ significantly from that produced by the suprathreshold stimulation. In some animals, two pathways were used, and this pairing-induced

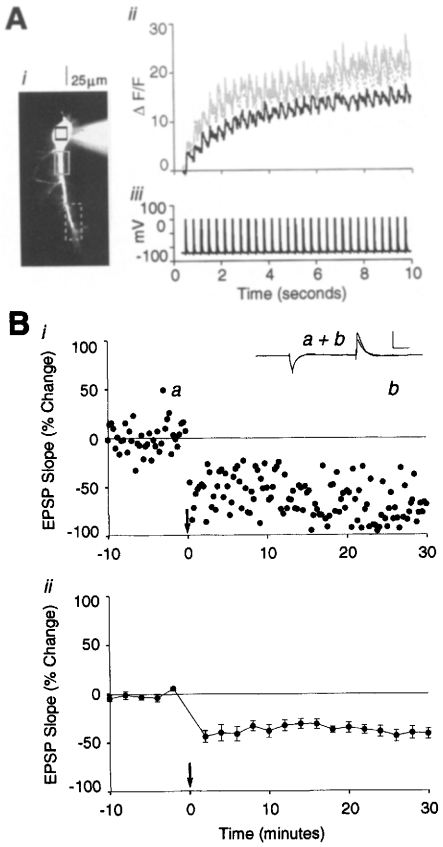

Figure 5: Pairing subthreshold EPSPs and somatic current injections that trigger action potentials at 3 Hz results in robust homosynaptic LTD. (*Ai*) Positive photomicrograph of a fura-2-filled CA1 pyramidal neuron from which data in *A* and *Bi* are presented. (*Aii*) Changes in fluorescence induced by pairing 10 mV EPSPs and depolarizing current injections (*Aiii*). The current pulses elicited a single action potential that occurred near the peak of each EPSP. (*Bi*) Averaged responses from four slices that received the pairing stimuli. EPSPs recorded from a single CA1 pyramidal cell prior to and following the application of the paired conditioning stimuli. (*Bi*) Single evoked responses obtained (*a*) prior to and (*b*) following the application of the pairing paradigm. (*Bii*) Averaged responses from four slices administered the pairing stimuli. Arrows indicate time points at which the conditioning stimulation was administered. Scale bars: 10 mV, 100 ms.

LTD, like that induced by suprathreshold synaptic stimulation, was found to be input specific ($n=3$). As with the suprathreshold and the somatic depolarization conditioning trains, large Ca^{2+} transients were also observed in response to the paired stimulation (soma: 17.6±2.5% ΔF/F; proximal: 21.1±1.6% ΔF/F; mid-radiatum: 16.1±2.5% ΔF/F; Fig. 5A).

Attempts to induce LTD by either administering synaptic stimulation while holding cells slightly depolarized (−45 to −50 mV) in voltage clamp (19.1±16.9%; $n=2$), or in conjunction with hyperpolarizing (rather than depolarizing) pulses all failed. In all of these cells, action potentials were not elicited during the conditioning stimulation (data not shown), and LTD was not induced.

ROLE OF NMDARs AND VGCCs IN HOMOSYNAPTIC LTD

Having established that LTD can be reliably induced by pairing synaptic activity with back-propagating action potentials, we next examined the involvement of NMDA receptors in this LTD. Conditioning stimuli administered in the presence of 50 μM APV failed to induce depression of evoked responses (15.8±13.3%; $n=6$; $P>0.05$; Fig. 6). Surprisingly, the Ca^{2+} transients in APV tended to be significantly smaller than those observed in normal ACSF ($P<0.05$; Fig. 6C) in each of the three regions of the neuron. To further evaluate this effect, 5-sec trains of 3-Hz stimuli were administered prior to the addition of APV in two neurons. These short trains did not alter evoked responses by themselves, but when APV was added to the ACSF and the trains were re-administered, Ca^{2+} transients were found to be reduced in all three regions (Pre: soma = 16.7±0.4%; proximal = 15.9±3.0%; mid-radiatum = 11.4±4.3%; Post: soma = 10.0±0.7%; proximal = 11.1±2.1%; mid-radiatum = 8.1±4.1%). Thus, NMDA receptors appear to be important for the induction of homosynaptic LTD and can greatly influence the magnitude of the Ca^{2+} transients recorded during the 3-Hz stimulation.

Heterosynaptic LTD in the CA1 region can be blocked by NMDA receptor antagonists and by antagonists of L-type VGCCs (Abraham and Wickens 1991). These findings have led to the suggestion that the activation of NMDA receptors may serve to prolong the period of postsynaptic depolarization and aid in the activation of L-type VGCCs. In addition, Hell et al. (1996) have shown that NMDA receptor activation in CA1 cells may persistently increase Ca^{2+} influx through L-type channels following intense synaptic activity (also see Chetkovich et al. 1991). To determine if homosynaptic LTD is also dependent on L-type VGCC activity, nimodipine (10 μM), a relatively nonphotolabile

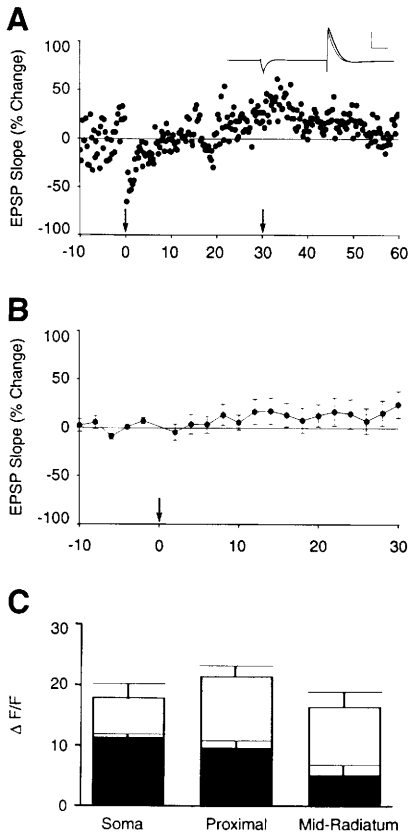

Figure 6: Blockade of NMDA-receptors prevents the induction of LTD with pairing stimulation. (*A*) Single evoked responses obtained from a neuron which was first administered suprathreshold stimulation (first arrow) and then pairing stimulation (second arrow) in the presence of (50 μM). (*B*) Average change in EPSP responses for slices ($n=6$) that were administered the 3-Hz pairing stimuli in the presence of APV. Note the similarity between the post-conditioning responses observed here, and those recorded when somatic current injections were administered at 3 Hz alone (Fig. 4). (C) Only moderate changes in fluorescence were observed during the application of the pairing stimuli in the presence of APV (solid bars) when compared with those observed in normal ACSF (open bars). The change in ΔF/F recorded during the conditioning trains was significantly reduced in all regions of the cell in APV as compared with control conditions. Scale bars: 10 mV, 100 ms.

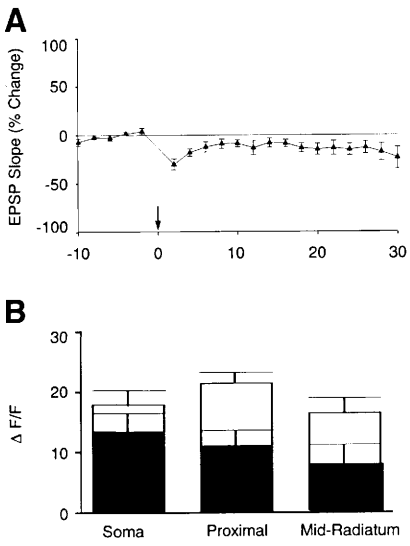

Figure 7: Nimodipine, an L-type VGCC antagonist, prevents the induction of pairing- induced LTD. (*A*) Application of paired conditioning stimulation (arrow) produced LTD of recorded EPSPs in the presence of (10 μM; $-23.4\pm11.0\%$; $n=5$). The magnitude of this depression was attenuated severely when compared with that normally observed following the application of the 3-Hz stimuli ($-49.7\pm5.1\%$; $P<0.05$; Fig. 5). (*B*) Changes in fluorescence recorded in the soma ($13.4\pm3.1\%$), proximal ($11.0\pm2.9\%$), and mid-radiatum ($8\pm3.1\%$) dendrites were also reduced significantly ($P<0.05$) when compared with those recorded in normal ACSF (open bars; data from Fig. 6).

dihydropyridine antagonist, was added to the perfusion medium 15 min prior to application of the low-frequency conditioning stimuli. The change in neuronal $[Ca^{2+}]_i$ was reduced significantly when the transients obtained in nimodipine were compared with those observed with the pairing paradigm in normal ACSF (Fig. 7B). Although a small depression was observed following the induction procedure, a significant degree of LTD was not apparent at 30 min post-conditioning ($-7.9\pm8.7\%$; $n=5$; $P>0.05$; Fig. 7).

As mentioned previously, it is known that the contribution of L-type VGCCs to optically measured Ca^{2+} transients diminishes with increasing distance from the soma (Christie et al. 1995). Instead, VGCCs sensitive to Ni^{2+} tend to be the major contributor to Ca^{2+} transients in more distal dendritic regions (Christie et al. 1995; Magee et al. 1995). To test the potential involvement of R- and T-type channels in LTD, $NiCl_2$ (25 μM) was added to the bathing medium 5–10 min prior to low-frequency conditioning and then washed out at the end of the stimulus train. LTD was not induced in these neurons ($7.7\pm8.9\%$; $n=5$), and an examination of the Ca^{2+} transients recorded during the conditioning stimulation revealed them to be significantly reduced as compared with control recordings (Fig. 8).

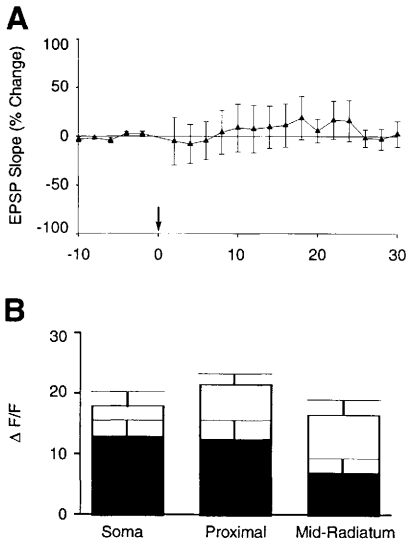

Figure 8: Blockade of LTD by $NiCl_2$. (*A*) Blockade of R- and T-type VGCCs with Ni^{2+} (25 μM) prevented the induction of LTD. (*B*) Changes in fluorescence recorded in the soma (13.2±3.6%), proximal (12.7±3.0%), and mid-radiatum (7.2±1.9%) dendrites were significantly reduced ($P<0.05$) when compared with those recorded in normal ACSF (open bars; Fig.6).

Discussion

The present results indicate that back-propagating action potentials occurring in conjunction with 3-Hz EPSPs can increase dramatically the efficacy for induction of homosynaptic LTD at Schaffer collateral–CA1 pyramidal neuron synapses. Under our experimental conditions, synaptic stimulation that was subthreshold for action potentials did not induce LTD, although a short-lasting depression (5–10 min) was sometimes observed. Many of the subthreshold EPSPs were actually quite large, sometimes >15 mV in amplitude, yet they failed to exhibit LTD, implying that T-channel activation by itself is not sufficient to induce LTD under these conditions. The generation of postsynaptic action potentials alone at 3 Hz was also not sufficient for LTD induction, although a weak potentiation that decayed over the course of the postconditioning recording period was often produced. In contrast, suprathreshold 3-Hz stimuli, which produced synaptically generated action potentials, produced robust LTD. Furthermore, subthreshold EPSPs that are usually unable to induce LTD by themselves, produced robust LTD when they were paired with postsynaptically generated action potentials. It appears, therefore, that the presence of back-propagating action potentials, rather than the stronger presynaptic stimulation, may be a critical parameter for LTD induction. Although postsynaptic action potentials may not be an absolute requirement for LTD induction (Neveu and Zucker 1996), they may play an important facilitatory role under normal physiological conditions.

The LTD induced by our stimulation protocols was blocked by the NMDAr antagonist APV, and was also severely reduced or prevented by the VGCC blockers nimodipine and Ni^{2+}. Changes in $[Ca^{2+}]_i$ during the LTD induction protocols were monitored in the soma, proximal dendrites, and mid-radiatum regions of CA1 neurons. As expected from previous work, only small increases in $[Ca^{2+}]_i$ were observed in these regions during subthreshold synaptic stimulation, but large increases in $[Ca^{2+}]_i$ occurred in all three regions in response to action potentials. A rather surprising finding was that for each of the three agents that blocked or reduced the induction of LTD, there were concomitant reductions in the rises in $[Ca^{2+}]_i$ during the 3-Hz stimulations. One possible conclusion from these results is that a reduction in Ca^{2+} entry, whether this occurs as a result of a reduction in $[Ca^{2+}]_i$ through either NMDA receptors, L-type VGCCs, or R- and T- type VGCCs, reduces or blocks LTD induction. The fact that APV reduced the synaptically induced Ca^{2+} transients in the soma as well as the dendrites would suggest that APV, in addition to blocking Ca^{2+} entry through NMDArs, also reduces the NMDAr-mediated depolarization and secondary Ca^{2+} entry through VGCCs, as has been suggested previously (Miyakawa et al. 1992; Regehr and Tank 1992). It may also be that Ca^{2+} entry in multiple locations of the neuron is necessary for LTD induction.

Ca^{2+} entry into dendritic spines of hippocampal pyramidal neurons through VGCCs has been shown recently by several authors (Jaffe et al. 1994; Segal 1995; Yuste and Denk 1995). Yuste and Denk (1995) showed a supralinear summation of Ca^{2+} signals in spines when action potentials and EPSPs occurred simultaneously and this supralinear summation was reduced with APV (R. Yuste, D.W. Tank, and W. Denk, pers. comm). One possible explanation for the results presented here is that the LTD is dependent on this large Ca^{2+} influx into spines, which occurs only when synaptically generated EPSPs and back-propagating spikes occur simultaneously in the spine (Yuste and Denk 1995). Thus, this supralinear summation can be prevented with APV, nimodipine, or Ni^{2+}, and

the synapse will fail to exhibit LTD under these conditions.

Previous research has shown that the L-, R-, and T-subtype of Ca^{2+} channels appear throughout the soma and dendrites of CA1 cells (Christie et al. 1995; Magee and Johnston 1995; Magee et al. 1995). Voltage-gated Ca^{2+} channels of the L-subtype appear to be more clustered around the soma and proximal dendrites of these cells (Westenbroek et al. 1990; Magee and Johnston 1995), while T- and R-subtypes are the main contributors to Ca^{2+}-entry in the more distal (>50 μm) dendritic regions (Christie et al. 1995; Magee et al. 1995; Magee and Johnston 1995). In addition, the contribution of these channels to synaptic plasticity does not seem restricted to the LTD observed here, as Ni^{2+} sensitive channels have also been reported to play a role in LTP (Chen et al. 1995). As there is yet no specific blocker for either the T- or R-type VGCC, it is unclear which channel is important for synaptic plasticity, or if both play a role. The role of the L-type channel in LTD is even more controversial, with some groups reporting a block with nimodipine in young (3- to 7-day-old) rats (Bolshakov and Siegelbaum 1994), whereas other groups reported no effect (Selig et al. 1995). Differences in induction procedures, developmental factors, or the location of stimulating electrodes may all contribute to the different results.

It should be emphasized that the results reported here, namely the importance of back-propagating action potentials for LTD induction and the fact that both NMDAr antagonists and VGCC blockers reduced or blocked LTD, were from synapses along the first 100–200 μm of the apical dendrites and that more distal synapses may follow different rules for induction. Such a possibility is entirely consistent with recent findings concerning the nonhomogeneous properties of the apical dendrites. For example, the amplitude of back-propagating action potentials is not constant with distance out the dendrites (Callaway and Ross 1995; Spruston et al. 1995b); dendritic action potentials fail to propagate at more distal branch points (Spruston et al. 1995b); and the distribution of VGCC subtypes varies among regions of dendrites (Christie et al. 1995; Johnston et al. 1996; Magee and Johnston 1995). Different parts of dendrites appear to have different properties, and, thus, mechanisms of synaptic integration and plasticity that depend on these properties may not generalize from one area of the dendrites to another. Nevertheless, back-propagating action potentials can provide a powerful feedback signal from the soma to the synapses to indicate, through a depolarization and subsequent Ca^{2+} influx, that an output of the neuron has occurred. Such a feedback signal is ideally suited for one component of Hebbian and non-Hebbian learning mechanisms.

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References

Abraham, W.C. and J.R. Wickens. 1991. Heterosynaptic long-term depression is facilitated by blockade of inhibition in area CA1 of the hippocampus. *Brain Res.* **546:** 336–340.

Bolshakov, V.Y. and S.A. Siegelbaum. 1994. Postsynaptic induction and presynaptic expression of hippocampal long-term depression. *Science* **264:** 1148–1152.

Callaway, J.C. and W.N. Ross. 1995. Frequency dependent propagation of sodium action potentials in dendrites of hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* **74:** 1395–1403.

Chen, Z.X., S. Fujii, K.I. Ito, H. Kato, K. Kaneko, and H. Miyakawa. 1995. Activation of dopamine D1 receptors enhances long-term depression of synaptic transmission induced by low frequency stimulation in rat hippocampal CA1 neurons. *Neurosci. Lett.* **188:** 195–198.

Chetkovich, D., R. Gray, D. Johnston, and J.D. Sweatt. 1991. *N*-methyl-D-aspartate receptor activation increases cAMP levels and voltage-gated Ca^{2+} channel activity in area CA1 of hippocampus. *Proc. Natl. Acad. Sci.* **88:** 6467–6471.

Christie, B.R., D.S. Kerr, and W.C. Abraham. 1994. Flip side of synaptic plasticity: Long-term depression mechanisms in the hippocampus. *Hippocampus* **4:** 127–135.

Christie, B.R., L.S. Eliot, K.I. Ito, H. Miyakawa, and D. Johnston. 1995. Different Ca^{2+} channels in soma and dendrites of hippocampal pyramidal neurons mediate spike-induced Ca^{2+} influx. *J. Neurophysiol.* **73:** 2553–2557.

Christofi, G., A.V. Nowicky, S.R. Bolsover, and L.J. Bindman. 1993. The postsynaptic induction of non-associative long-term depression of excitatory synaptic

transmission in rat hippocampal slices. *J. Neurophysiol.* **69(1):** 219–229.

Dudek, S.M. and M.F. Bear. 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. *Proc. Natl. Acad. Sci.* **8:** 4363–4367.

———. 1993. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J. Neurosci.* **13:** 291–2918.

Hell, J.W., R.E. Westenbroek, L.J. Breeze, K.K.W. Wang, C. Chavkin, and W.A. Catterall. 1996. *N*-methyl-D-aspartate receptor-induced proteolytic conversion of postsynaptic class C L-type calcium channels in hippocampal neurons. *Proc. Natl. Acad. Sci.* **93:** 3362–3367.

Jaffe, D.B., D. Johnston, N. Lasser-Ross, J.E. Lisman, H. Miyakawa, and W.N. Ross. 1992. The spread of Na^+ spikes determines the pattern of dendritic Ca^{2+} entry into hippocampal neurons. *Nature* **357:** 244–246.

Jaffe, D.B., S.A. Fisher, and T.H. Brown. 1994. Confocal laser scanning microscopy reveals voltage-gated calcium signals within hippocampal dendritic spines. *J. Neurobiol.* **25:** 220–233.

Johnston, D., J.C. Magee, C.M. Colbert, and B.R. Christie. 1996. Active properties of neuronal dendrites. *Annu. Rev. Neurosci.* **19:**165–186.

Kullmann, D.M., D.J. Perkel, T. Manabe, and R.A. Nicoll. 1992. Ca^{2+} entry via postsynaptic voltage-sensitive Ca^{2+} channels can transiently potentiate excitatory synaptic transmission in the hippocampus. *Neuron* **9:** 1175–1183.

Larkman, A.U. and J.J.B. Jack. 1995. Synaptic plasticity: Hippocampal LTP *Curr. Opin. Neurobiol.* **5:** 324–334.

Linden, D.J. and J.A. Connor. 1995. Long-term synaptic depression. *Annu. Rev. Neurosci.* **18:** 319–357.

Magee, J.C. and D. Johnston. 1995. Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *J. Physiol.* (*London*) **487:** 67–90.

Magee, J.C., G. Christofi, H. Miyakawa, B. Christie, N. Lasser-Ross, and D. Johnston. 1995. 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.

Mayer, M.L. and G.L. Westbrook. 1987. Permeation and block of *N*-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *J. Physiol.* (*London*) **394:** 501–527.

Miyakawa, H., W.N. Ross, D. Jaffe, J.C. Callaway, N. Lasser-Ross, J.E. Lisman, and D. Johnston. 1992. Synaptically activated increases in Ca^{2+} concentration in hippocampal CA1 pyramidal cells are primarily due to voltage-gated Ca^{2+} channels. *Neuron* **9:** 1163–1173.

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

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

Regehr, W.G. and D.W. Tank. 1990. Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. *Nature* **345:** 807–810.

———. 1992. Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. *J. Neurosci.* **12:** 4202–4223.

Segal, M. 1995. Fast imaging of $[Ca^{2+}]_i$ reveals presence of voltage-gated calcium channels in dendritic spines of cultured hippocampal neurons. *J. Neurophysiol.* **74:** 484–488.

Selig, D.K., H.K. Lee, M.F. Bear, and R.C. Malenka. 1995. Reexamination of the effects of MCPG on hippocampal LTP, LTD, and depotentiation. *J. Neurophysiol.* **74:** 1075–1082.

Spruston, N., P. Jonas, and B. Sakmann. 1995a. Dendritic glutamate receptor channels in rat hippocampal CA3 and CA1 pyramidal neurons. *J. Physiol.* (*London*) **482:** 325–352.

Spruston, N., Y. Schiller, G. Stuart, and B. Sakmann. 1995b. Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. *Science* **268:** 297–300.

Westenbroek, R.E., M.K. Ahlijanian, and W.A. Catterall. 1990. Clustering of L-type Ca^{2+} channels at the base of major dendrites in hippocampal pyramidal neurons. *Nature* **347:** 281–284.

Wexler, E.M. and P.K. Stanton 1993. Priming of homosynaptic long-term depression in hippocampus by previous synaptic activity. *NeuroReport* **4:** 591–594.

Yuste, R. and W. Denk. 1995. Dendritic spines as basic functional units of neuronal integration. *Nature* **375:** 682–684.

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